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(72) Inventor(s):
Warren Roger Wong
Russell Greiner
Tim Alan Rosborough
Brent Allan Lefebvre
Noah Alexander Epstein
Jack Barless Newton
David Scott Wishart

(73) Proprietor(s):
Chenomx Inc
(Incorporated in Canada - Alberta)
Suite 800 Ironwood Profesional Centre,
10050 - 112 Street, EDMONTON,
Alberta T5K 2J1, Canada

(74) Agent and/or Address for Service:
Harrison Goddard Foote
Belgrave Hall, Belgrave Street, LEEDS,
LS2 8DD, United Kingdom

2410559

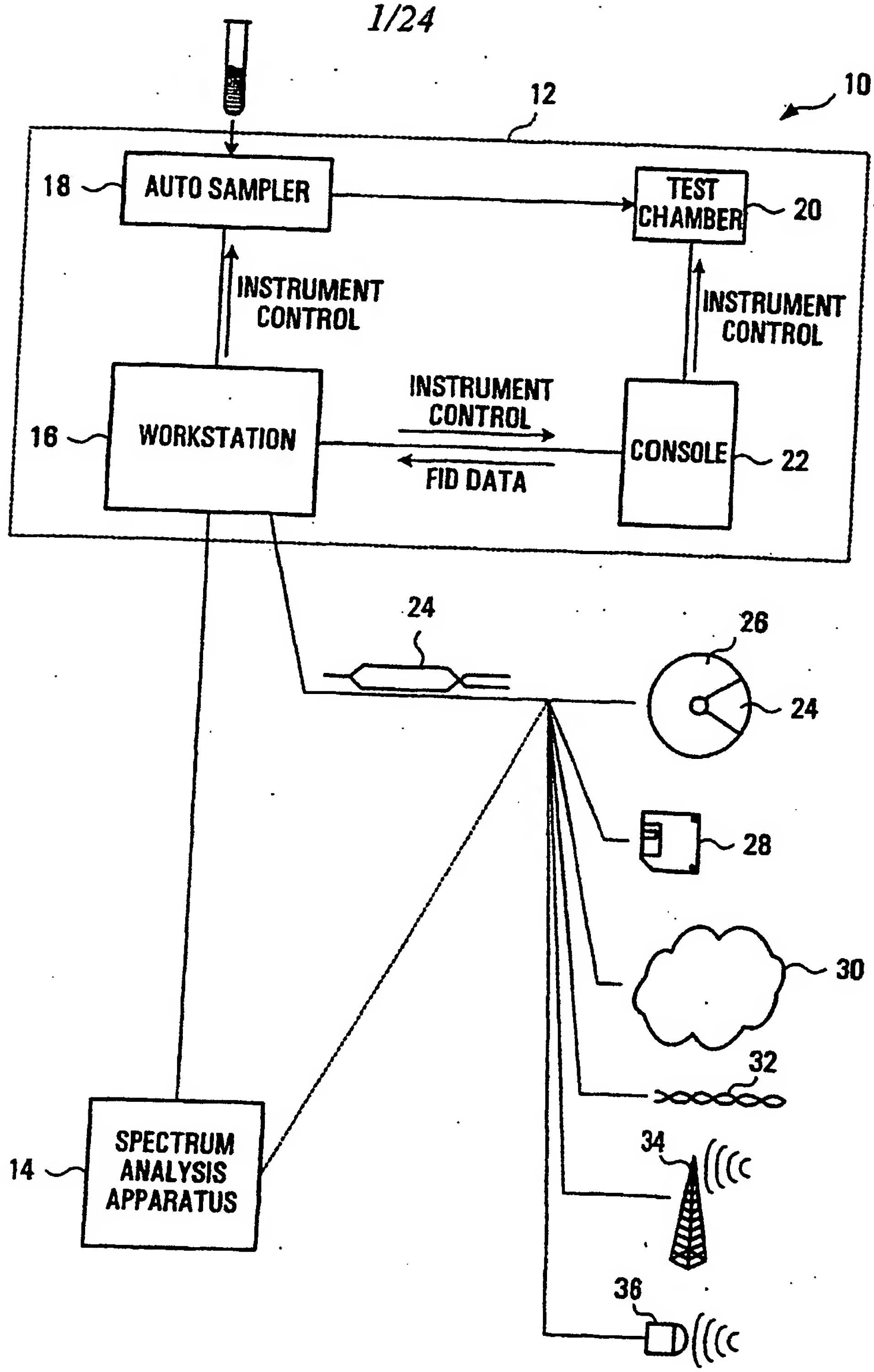


FIG. 1

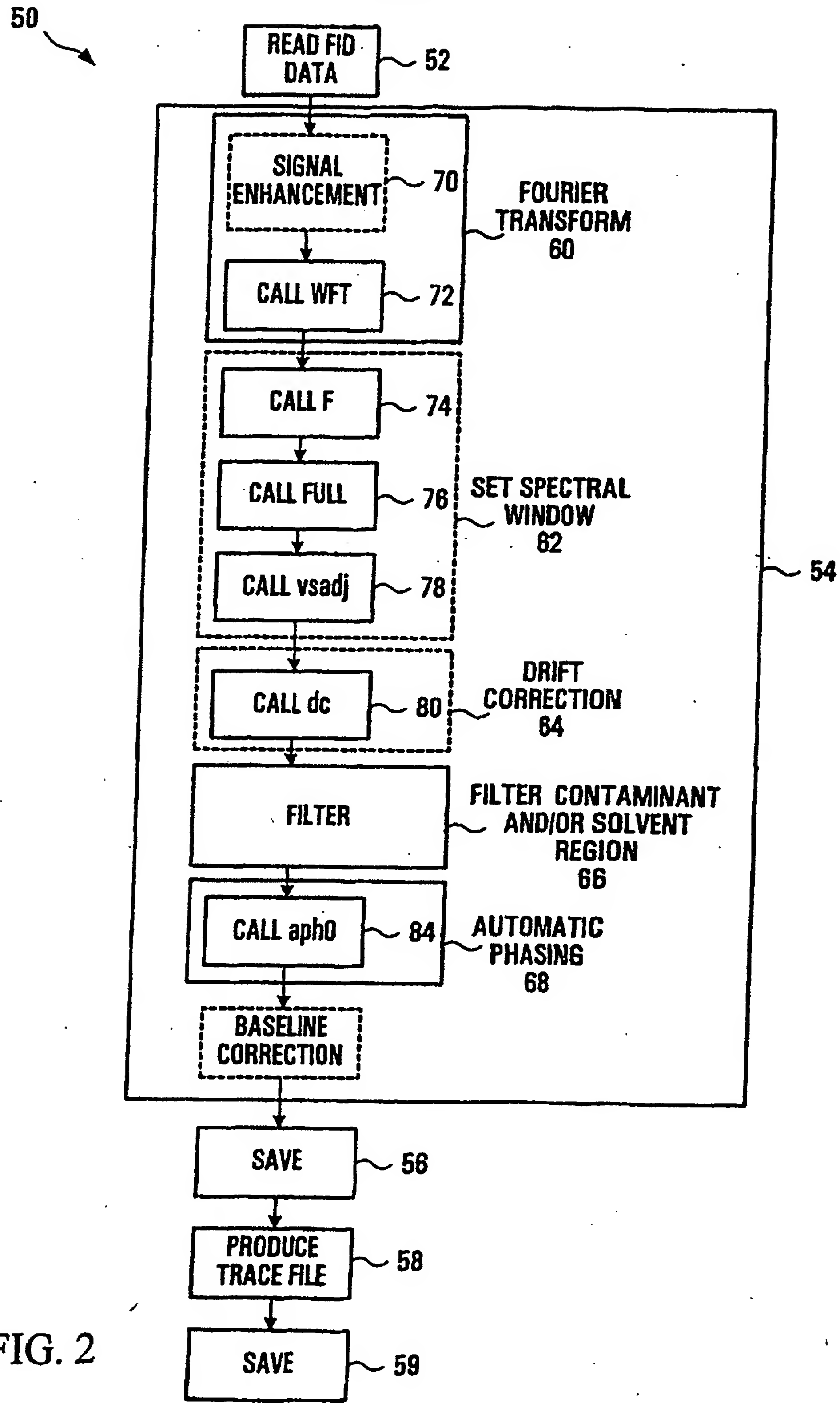
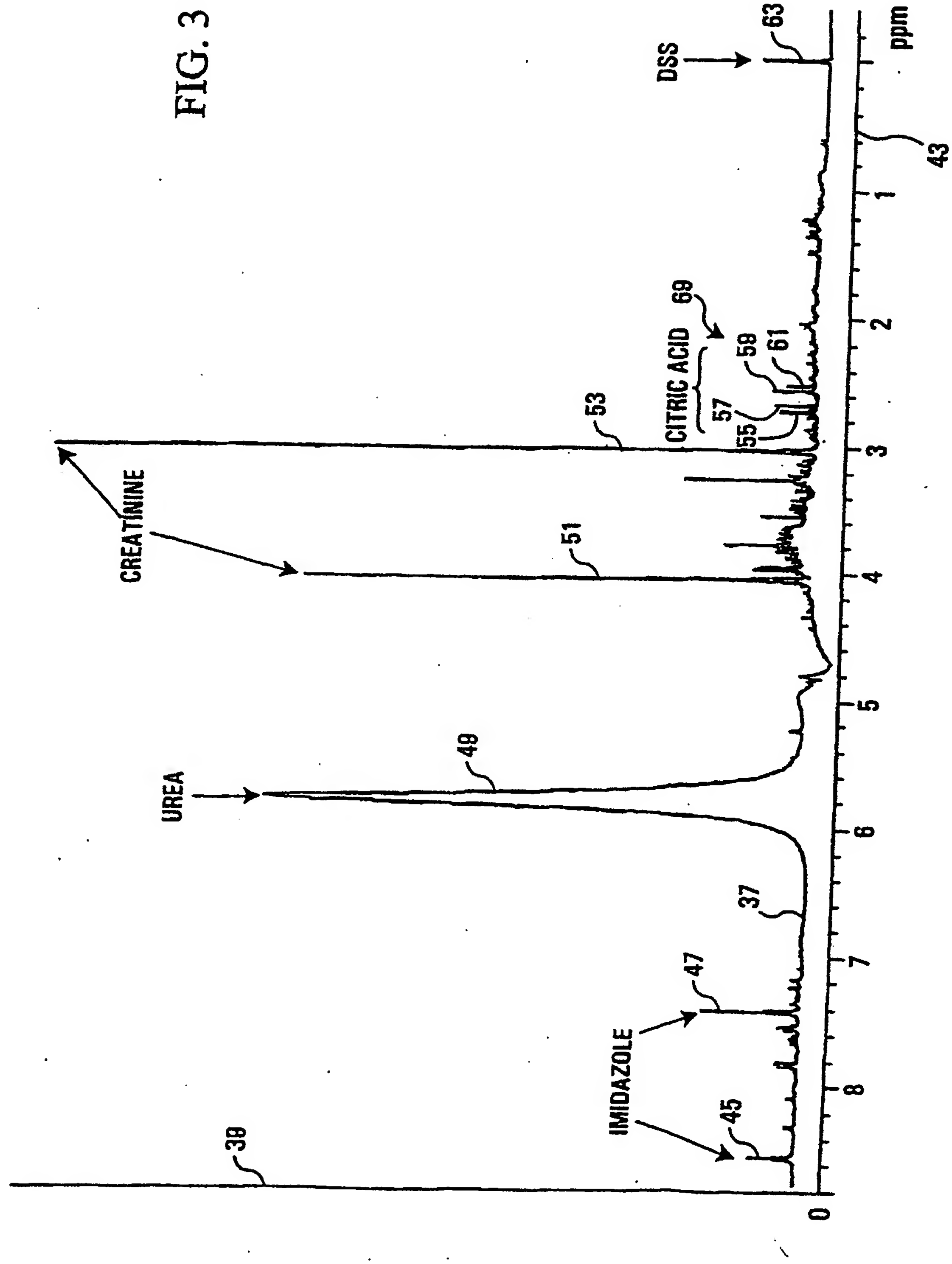

50
READ FID DATA
52
SIGNAL ENHANCEMENT
70
FOURIER TRANSFORM 60
CALL WFT
72
CALL F
74
CALL FULL
76
SET SPECTRAL WINDOW 62
CALL vsadj
78
54
CALL dc
80
DRIFT CORRECTION 64
FILTER
FILTER CONTAMINANT AND/OR SOLVENT REGION 66
CALL aph0
84
AUTOMATIC PHASING 68
BASELINE CORRECTION
SAVE
56
PRODUCE TRACE FILE
58
SAVE
59

FIG. 2

FIG. 3



39
IMIDAZOLE
45
47
37
UREA
49
CREATININE
51
53
CITRIC ACID
55
57
59
61
69
DSS
63
43
ppm
0
8
7
6
5
4
3
2
1

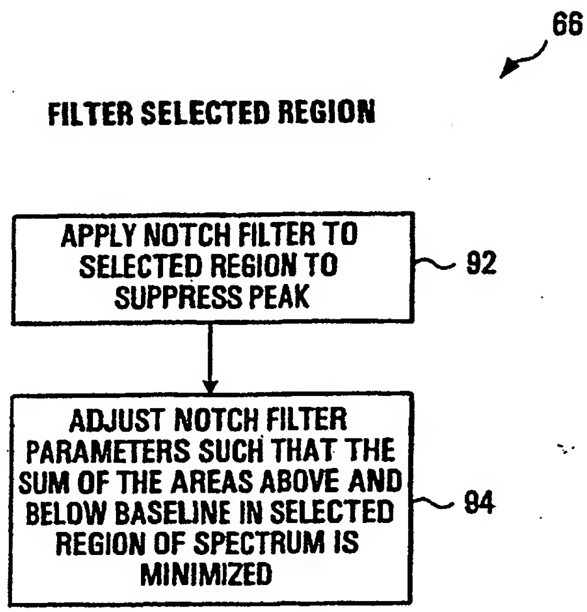

66
FILTER SELECTED REGION
APPLY NOTCH FILTER TO SELECTED REGION TO SUPPRESS PEAK
92
ADJUST NOTCH FILTER PARAMETERS SUCH THAT THE SUM OF THE AREAS ABOVE AND BELOW BASELINE IN SELECTED REGION OF SPECTRUM IS MINIMIZED
94

FIG. 4

PROCESS FOR IDENTIFYING COMPOUNDS FROM A TEST SPECTRUM

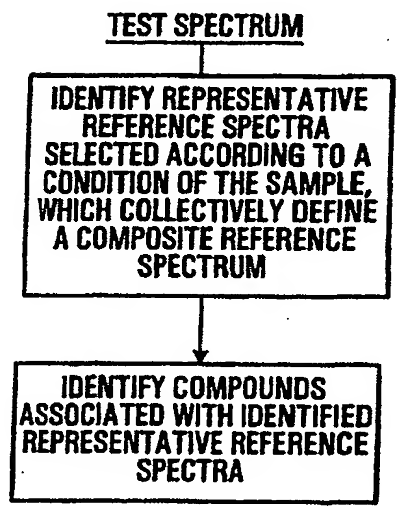
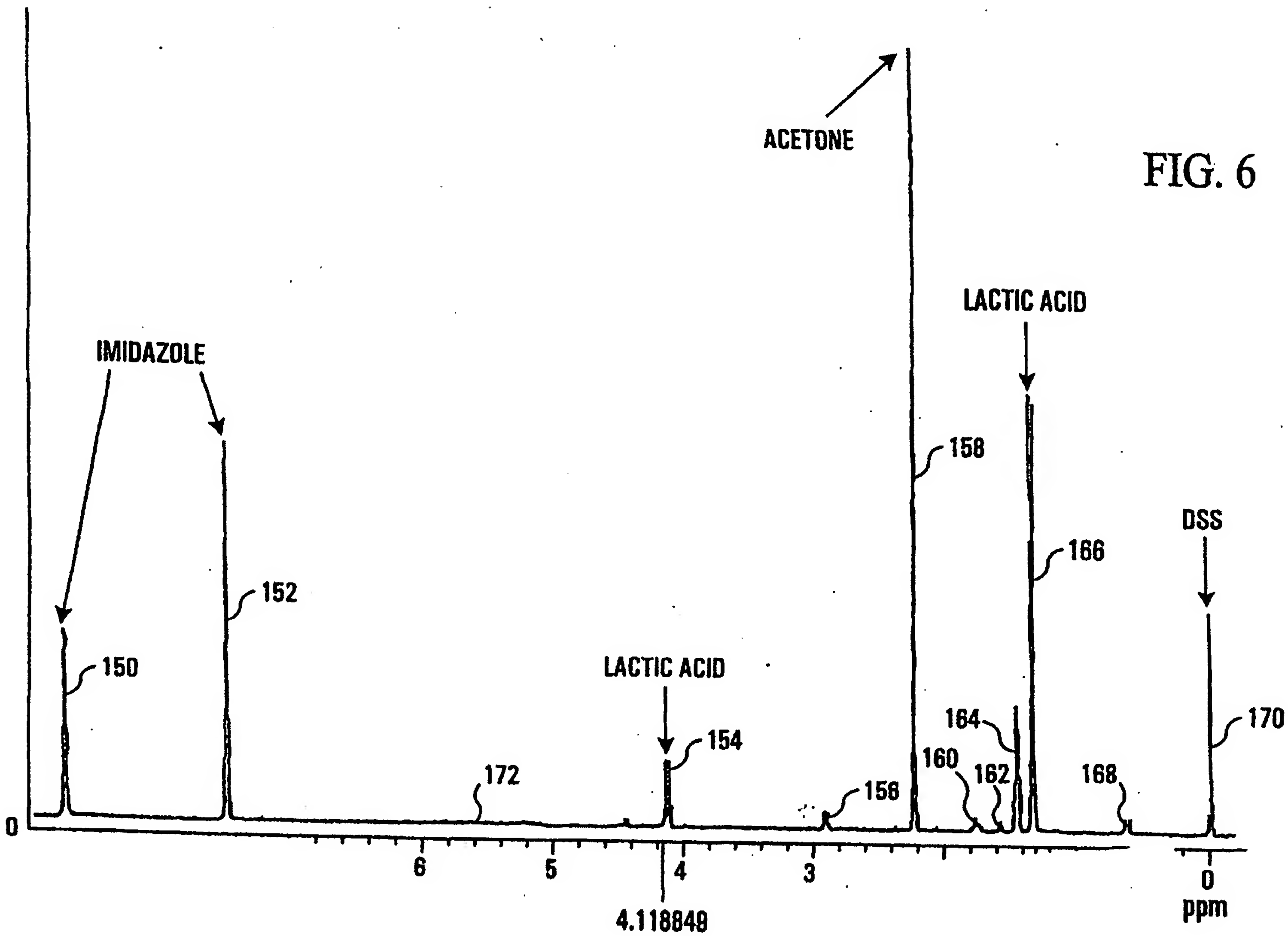


FIG. 5

FIG. 6


ACETONE
LACTIC ACID
IMIDAZOLE
158
166
DSS
152
150
LACTIC ACID
164
170
154
172
160
162
168
156
0
6
5
4
3
0
ppm
4.118849

REAL SPECTRAL DATA - pH=5.10


```
<?xml version="1.0"?>
<!--compoundDB version="2.0"-->
<compound>                                                           200
  <name>LacticAcid</name>
  <molecularWeight>90.08</molecularWeight>

  <experiment>
  <pH>5.1</pH>
  <temperatureKelvin>298.150000</temperatureKelvin>
  <DSSRatio>0.533668</DSSRatio>
  <concentration>4.308710</concentration>
  <timestamp>Sept 5 2001</timestamp>                                202
  <sourceFID>lacticacid.fid</sourceFID>
  <magnetMHz>400.120773</magnetMHz>
  <spectralWidthHz>6006.006006</spectralWidthHz>
</experiment>

<cluster>                                                     206
210 <protonNumber>3</protonNumber>
212 <quantification>1</quantification>
  <subCluster>
   <centerPPM>1.321559</centerPPM>
214 <lorentzianWidthAdjust>2.893258</lorentzianWidthAdjust>
   <peak>
220  <offsetCenterHz>-3.455480</offsetCenterHz>
222  <height>0.988680</height>                               216
224  <protonRatio>1.491461</protonRation>                            204
   </peak>
   <peak>
    <offsetCenterHz>3.455480</offsetCenterHz>
    <height>1.0000000</height>                               218
    <protonRation>1.508539</protonRatio>
   </peak>
  </subCluster>
</cluster>
```


FIG. 7A

208

```
<cluster>
  <protonNumber>1</protonNumber>
  <quantification>0</quantification>
  <subCluster>
    <centerPPM>4.118849</centerPPM>
    <lorentzianWidthAdjust>13.441432<lorentzianWidthAdjust>
    <peak>
      <offsetCenterHz>-10.366999</offsetCenterHz>
      <height>0.057244</height>
      <protonRatio>0.130861</protonRatio>
    </peak>
    <peak>
      <offsetCenterHz>-3.43221144</offsetCenterHz>
      <height>0.164751</height>
      <protonRatio>0.376629</protonRatio>
    </peak>
    <peak>
    <offsetCenterHz>3.487744</offsetCenterHz>
      <height>0.161840</height>
      <protonRatio>0.369975</protonRatio>
    </peak>
    <peak>
    <offsetCenterHz>10.366999</offsetCenterHz>
      <height>0.053601</height>
      <protonRatio>0.122535</protonRatio>
    </peak>
  </subCluster>
 </cluster>
</compound>
```

204

FIG. 7B

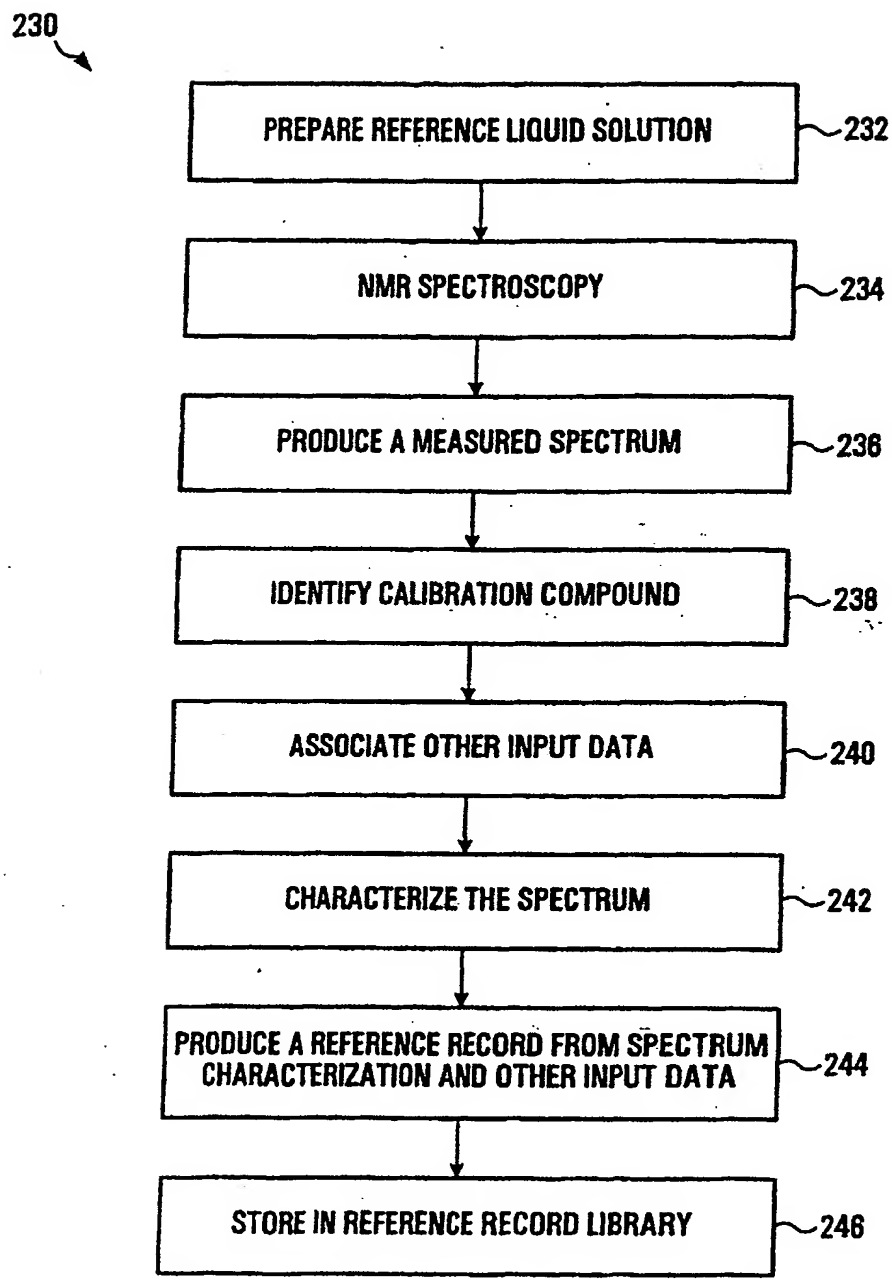

230
PREPARE REFERENCE LIQUID SOLUTION
232
NMR SPECTROSCOPY
234
PRODUCE A MEASURED SPECTRUM
236
IDENTIFY CALIBRATION COMPOUND
238
ASSOCIATE OTHER INPUT DATA
240
CHARACTERIZE THE SPECTRUM
242
PRODUCE A REFERENCE RECORD FROM SPECTRUM CHARACTERIZATION AND OTHER INPUT DATA
244
STORE IN REFERENCE RECORD LIBRARY
246

FIG. 8

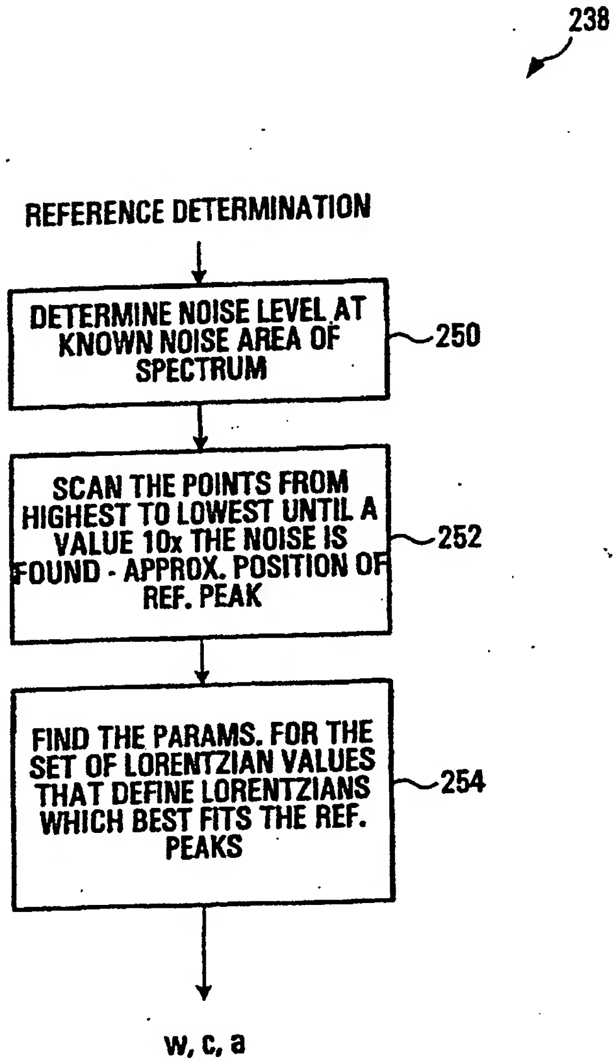

238
REFERENCE DETERMINATION
DETERMINE NOISE LEVEL AT KNOWN NOISE AREA OF SPECTRUM
250
SCAN THE POINTS FROM HIGHEST TO LOWEST UNTIL A VALUE 10x THE NOISE IS FOUND - APPROX. POSITION OF REF. PEAK
252
FIND THE PARAMS. FOR THE SET OF LORENTZIAN VALUES THAT DEFINE LORENTZIANS WHICH BEST FITS THE REF. PEAKS
254
w, c, a

FIG. 9

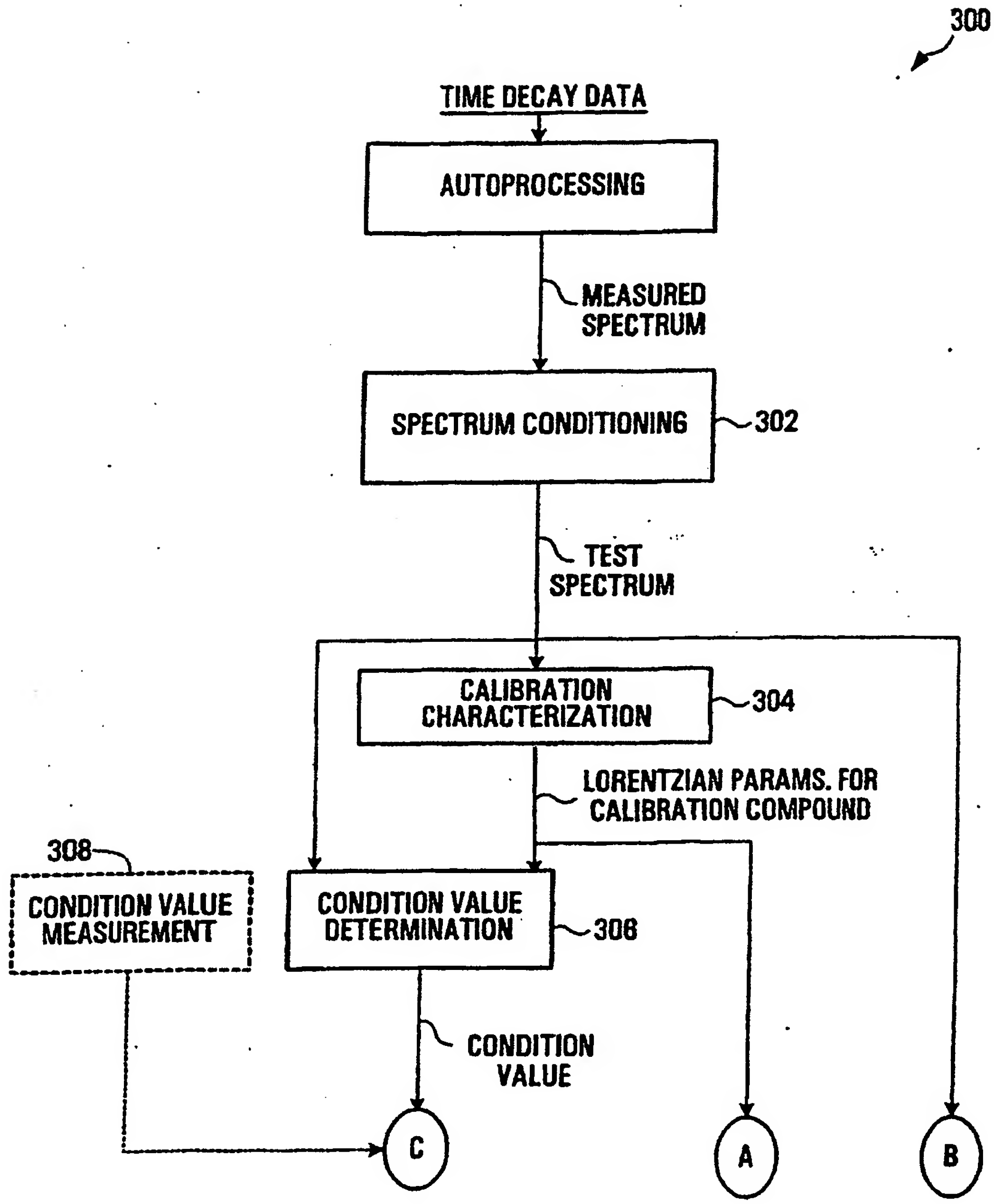

300
TIME DECAY DATA
AUTOPROCESSING
MEASURED SPECTRUM
SPECTRUM CONDITIONING
302
TEST SPECTRUM
CALIBRATION CHARACTERIZATION
304
LORENTZIAN PARAMS. FOR CALIBRATION COMPOUND
308
CONDITION VALUE MEASUREMENT
CONDITION VALUE DETERMINATION
306
CONDITION VALUE
C
A
B

FIG. 10A

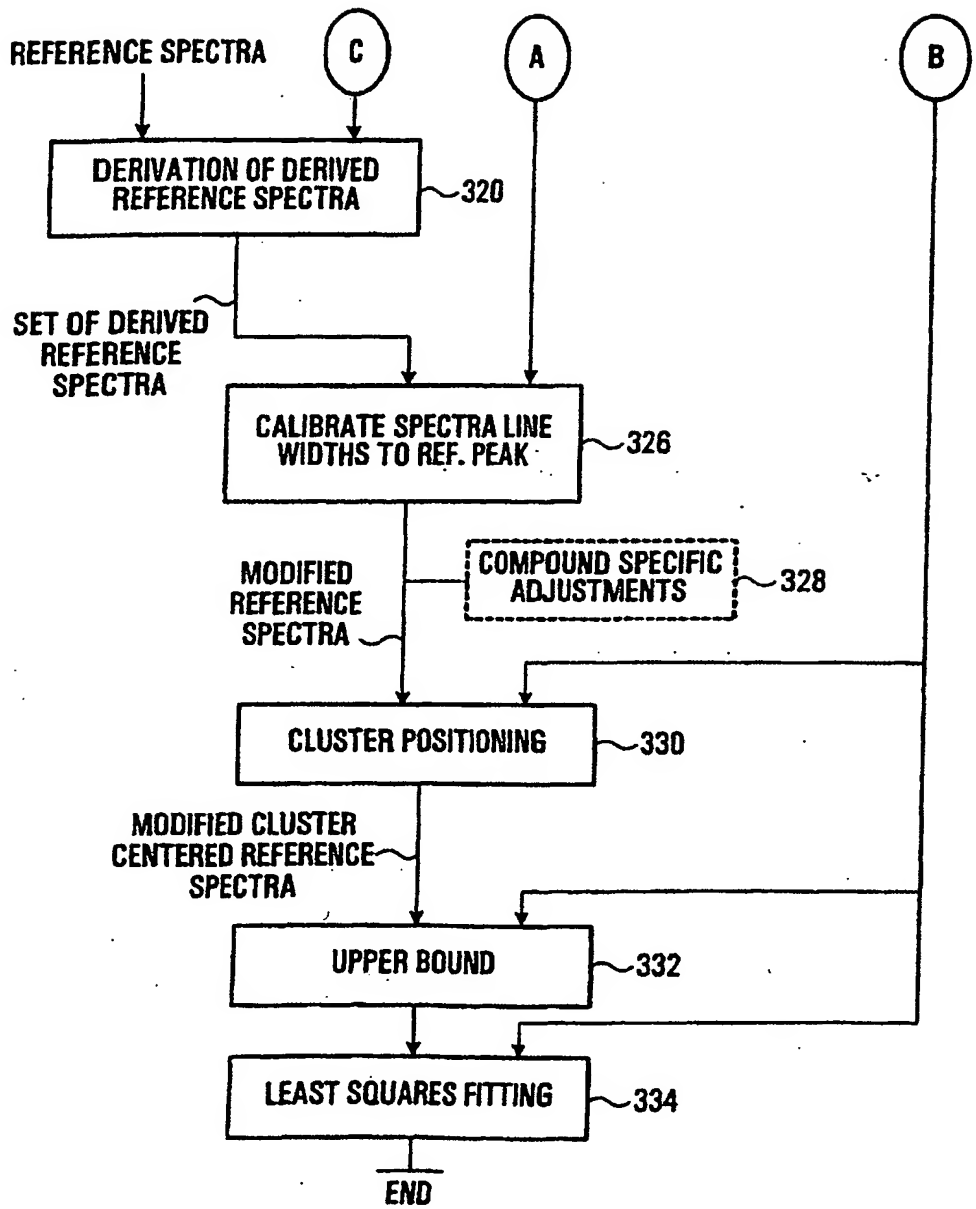

REFERENCE SPECTRA
C
A
B
DERIVATION OF DERIVED REFERENCE SPECTRA
320
SET OF DERIVED REFERENCE SPECTRA
CALIBRATE SPECTRA LINE WIDTHS TO REF. PEAK
326
MODIFIED REFERENCE SPECTRA
COMPOUND SPECIFIC ADJUSTMENTS
328
CLUSTER POSITIONING
330
MODIFIED CLUSTER CENTERED REFERENCE SPECTRA
UPPER BOUND
332
LEAST SQUARES FITTING
334
END

FIG. 10B

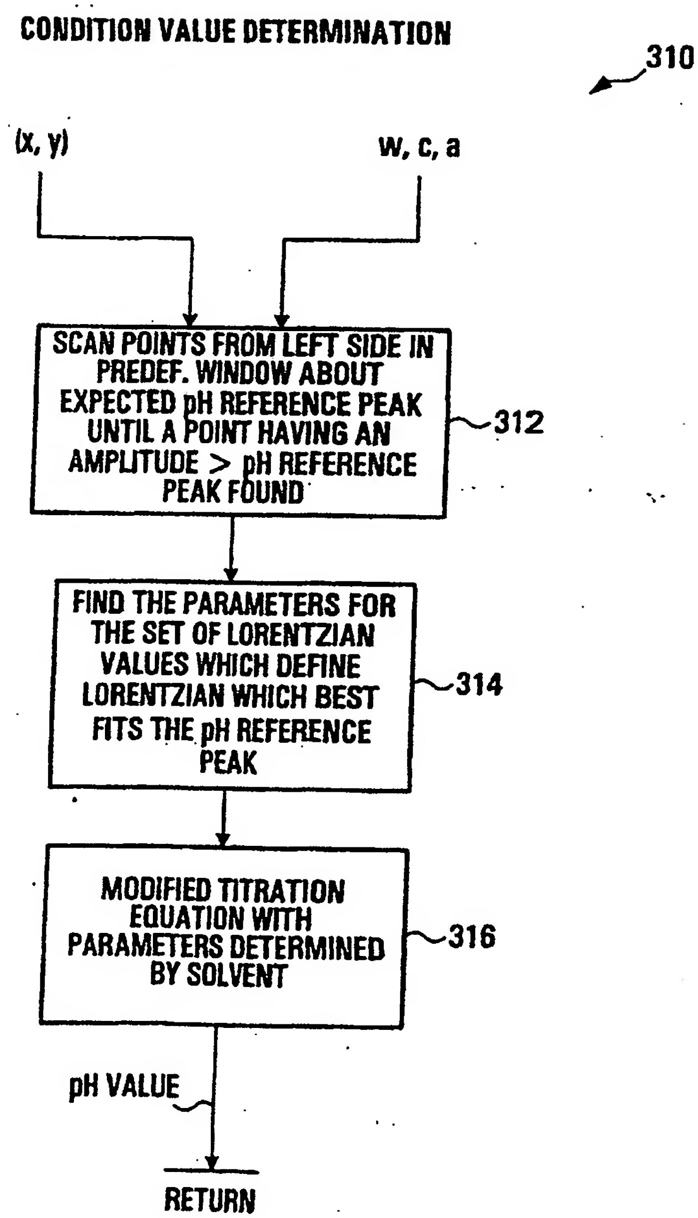

CONDITION VALUE DETERMINATION
310
(x, y)
w, c, a
SCAN POINTS FROM LEFT SIDE IN PREDEF. WINDOW ABOUT EXPECTED pH REFERENCE PEAK UNTIL A POINT HAVING AN AMPLITUDE > pH REFERENCE PEAK FOUND
312
FIND THE PARAMETERS FOR THE SET OF LORENTZIAN VALUES WHICH DEFINE LORENTZIAN WHICH BEST FITS THE pH REFERENCE PEAK
314
MODIFIED TITRATION EQUATION WITH PARAMETERS DETERMINED BY SOLVENT
316
pH VALUE
RETURN

FIG. 11

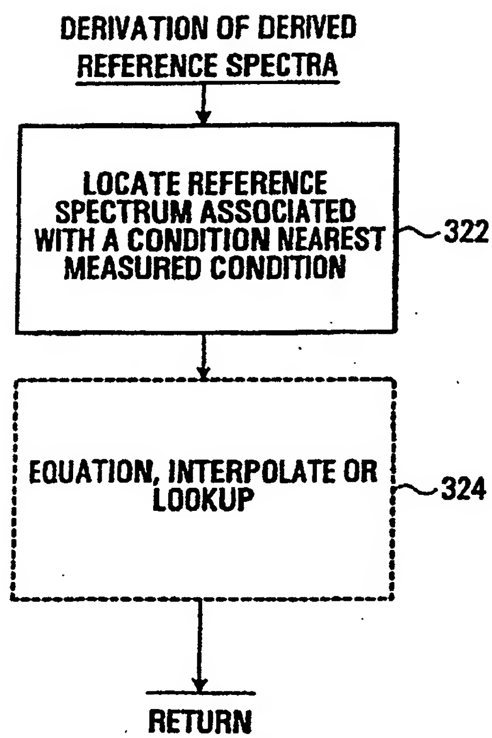

DERIVATION OF DERIVED REFERENCE SPECTRA
LOCATE REFERENCE SPECTRUM ASSOCIATED WITH A CONDITION NEAREST MEASURED CONDITION
322
EQUATION, INTERPOLATE OR LOOKUP
324
RETURN

FIG. 12

Real Spectral Data - pH=5.45


```
<?xml version="1.0"?>
<!-- compoundDB version="2.0" -->
<compound>
 <name>LecticAcid</name>
 <molecularWeight>90.08</molecularWeight>


 <experiment>
  <pH>5.45</pH>
  <temperatureKelvin>298.150000</temperatureKelvin>
  <DSSRatio>0.533668</DSSRatio>
  <concentration>4.308710</concentration>
  <timestamp>Sept 5 2001</timestamp>
  <sourceFID>lacticacid.fid</sourceFID>
  <magnetMHz>400.120699</magnetMHz>
  <spectralWidthHz>6006.006006</spectralWidthHz>
 </experiment>


 <cluster>
  <protonNumber>3</protonNumber>
  <quantification>1</quantification>
  <subCluster>
   <centerPPM>1.318770</centerPPM>
   <lorentzianWidthAdjust>1.562004</lorentzianWidthAdjust>
   <peak>
    <offsetCenterHz>-3.453329</offsetCenterHz>
    <height>0.983656</height>
    <protonRatio>1.487641</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>3.453329</offsetCenterHz>
    <height>1.000000</height>
    <protonRatio>1.512359</protonRatio>
   </peak>
  </subCluster>
 </cluster>
```


FIG. 13A

<cluster>
<protonNumber>1</protonNumber>
<quantification>0</quantification>
<subCluster>
<centerPPM>4.110990</centerPPM>
<lorentzianWidthAdjust>7.044502</lorentzianWidthAdjust>
<peak>
<offsetCenterHz>-10.371092</offsetCenterHz>
<height>0.054671</height>
<protonRatio>0.124148</protonRatio>
</peak>
<peak>
<offsetCenterHz>-3.448909</offsetCenterHz>
<height>0.164509</height>
<protonRatio>0.373572</protonRatio>
</peak>
<peak>
<offsetCenterHz>3.456819</offsetCenterHz>
<height>0.163879</height>
<protonRatio>0.372141</protonRatio>
</peak>
<peak>
<offsetCenterHz>10.371092</offsetCenterHz>
<height>0.057309</height>
<protonRatio>0.130139</protonRatio>
</peak>
</subCluster>
</cluster>
</compound>

FIG. 13B

Interpolated Data - pH=5.28


```
<?xml version="1.0"?>
<!-- compoundDB version="2.0" -->
<compound>
 <name>LacticAcid</name>
 <molecularWeight>90.08</molecularWeight>

 <experiment>
  <pH>5.45</pH>
  <temperatureKelvin>298.150000</temperatureKelvin> : copied
  <DSSRatio>0.533668</DSSRatio>
  <concentration>4.308710</concentration>
  <timestamp>Sept 5 2001</timestamp>
  <sourceFID>lacticacid.fid</sourceFID> :copied
  <magnetMHz>400.120699</magnetMHz>
  <spectralWidthHz>6006.006006</spectralWidthHz>
 </experiment>

 <cluster>
  <protonNumber>3</protonNumber>
  <quantification>1</quantification>
  <subCluster>
   <centerPPM>1.3202</centerPPM> : interpolated
   <lorentzianWidthAdjust>1.562004</lorentzianWidthAdjust>
   <peak>
    <offsetCenterHz>-3.453329</offsetCenterHz>
    <height>0.983656</height>
    <protonRatio>1.487641</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>3.453329</offsetCenterHz>
    <height>1.000000</height>
    <protonRatio>1.512359</protonRatio>
   </peak>
  </subCluster>
 </cluster>
```


FIG. 14A

```
<cluster>
  <protonNumber>1</protonNumber>
  <quantification>0</quantification>
  <subCluster>
   <centerPPM>4.1149</centerPPM> :interpolated
   <lorentzianWidthAdjust>7.044502</lorentzianWidthAdjust>
   <peak>
    <offsetCenterHz>-10.371092</offsetCenterHz>
    <height>0.054671</height>
    <protonRatio>0.124148</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>-3.448909</offsetCenterHz>
    <height>0.164509</height>
    <protonRatio>0.373572</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>3.456819</offsetCenterHz>
    <height>0.163879</height>
    <protonRatio>0.372141</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>10.371092</offsetCenterHz>
    <height>0.057309</height>
    <protonRatio>0.130139</protonRatio>
   </peak>
  </subCluster>
 </cluster>
</compound>
```


FIG. 14B

Generic Lactic Acid record


```
<?xml version="1.0"?>
<!-- compoundDB version="2.0" -->
<compound>
 <name>LacticAcid</name>
 <molecularWeight>90.08</molecularWeight>

 <experiment>
  <pH>5.45</pH>
  <temperatureKelvin>298.150000</temperatureKelvin>
  <DSSRatio>0.533668</DSSRatio>
  <concentration>4.308710</concentration>
  <timestamp>Sept 5 2001</timestamp>
  <sourceFID>lacticacid.fid</sourceFID>
  <magnetMHz>400.120899</magnetMHz>
  <spectralWidthHz>6006.006006</spectralWidthHz>
 </experiment>

 <cluster>
  <protonNumber>3</protonNumber>
  <quantification>1</quantification>
  <subCluster>
   <centerPPM>y=m1(pH)+b1</centerPPM>
   <lorentzianWidthAdjust>1.562004</lorentzianWidthAdjust>
   <peak>
    <offsetCenterHz>-3.453329</offsetCenterHz>
    <height>0.983656</height>
    <protonRatio>1.487641</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>3.453329</offsetCenterHz>
    <height>1.000000</height>
    <protonRatio>1.512359</protonRatio>
   </peak>
  </subCluster>
 </cluster>
```


FIG. 15A

```
<cluster>
  <protonNumber>1</protonNumber>
  <quantification>0</quantification>
  <subCluster>
   <centerPPM> y=m2(pH)+b2</centerPPM>
   <lorentzianWidthAdjust>7.044502</lorentzianWidthAdjust>
   <peak>
    <offsetCenterHz>-10.371092</offsetCenterHz>
    <height>0.054671</height>
    <protonRatio>0.124148</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>-3.448909</offsetCenterHz>
    <height>0.164509</height>
    <protonRatio>0.373572</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>3.456819</offsetCenterHz>
    <height>0.163878</height>
    <protonRatio>0.372141</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>10.371092</offsetCenterHz>
    <height>0.057309</height>
    <protonRatio>0.130139</protonRatio>
   </peak>
  </subCluster>
 </cluster>
</compound>
```


FIG. 15B

Generic Lactic Acid record with Lookup Table link


```
<?xml version="1.0"?>
<!-- compoundDB version="2.0" -->
<compound>
 <name>LacticAcid</name>
 <molecularWeight>90.08</molecularWeight>


 <experiment>
  <pH>5.45</pH>
  <temperatureKelvin>298.150000</temperatureKelvin>
  <DSSRatio>0.533668</DSSRatio>
  <concentration>4.308710</concentration>
  <timestamp>Sept 5 2001</timestamp>
  <sourceFID>lacticacid.fid</sourceFID>
  <magnetMHz>400.120699</magnetMHz>
  <spectralWidthHz>6006.006006</spectralWidthHz>
 </experiment>


 <cluster>
  <protonNumber>3</protonNumber>
  <quantification>1</quantification>
  <subCluster>
   <centerPPM>LOOKUP TABLE LINK1</centerPPM>
   <lorentzianWidthAdjust>1.562004</lorentzianWidthAdjust>
   <peak>
    <offsetCenterHz>-3.453329</offsetCenterHz>
    <height>0.983656</height>
    <protonRatio>1.487641</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>3.453329</offsetCenterHz>
    <height>1.000000</height>
    <protonRatio>1.512359</protonRatio>
   </peak>
  </subCluster>
 </cluster>
```


FIG. 16A

```
<cluster>
  <protonNumber>1</protonNumber>
  <quantification>0</quantification>
  <subCluster>
   <centerPPM> LOOKUP TABLE LINK2</centerPPM>
   <lorentzianWidthAdjust>7.044502</lorentzianWidthAdjust>
   <peak>
    <offsetCenterHz>-10.371092</offsetCenterHz>
    <height>0.054671</height>
    <protonRatio>0.124148</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>-3.448909</offsetCenterHz>
    <height>0.164509</height>
    <protonRatio>0.373572</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>3.456819</offsetCenterHz>
    <height>0.163879</height>
    <protonRatio>0.372141</protonRatio>
   </peak>
   <peak>
    <offsetCenterHz>10.371092</offsetCenterHz>
    <height>0.057309</height>
    <protonRatio>0.130139</protonRatio>
   </peak>
  </subCluster>
 </cluster>
</compound>
```


FIG. 16B

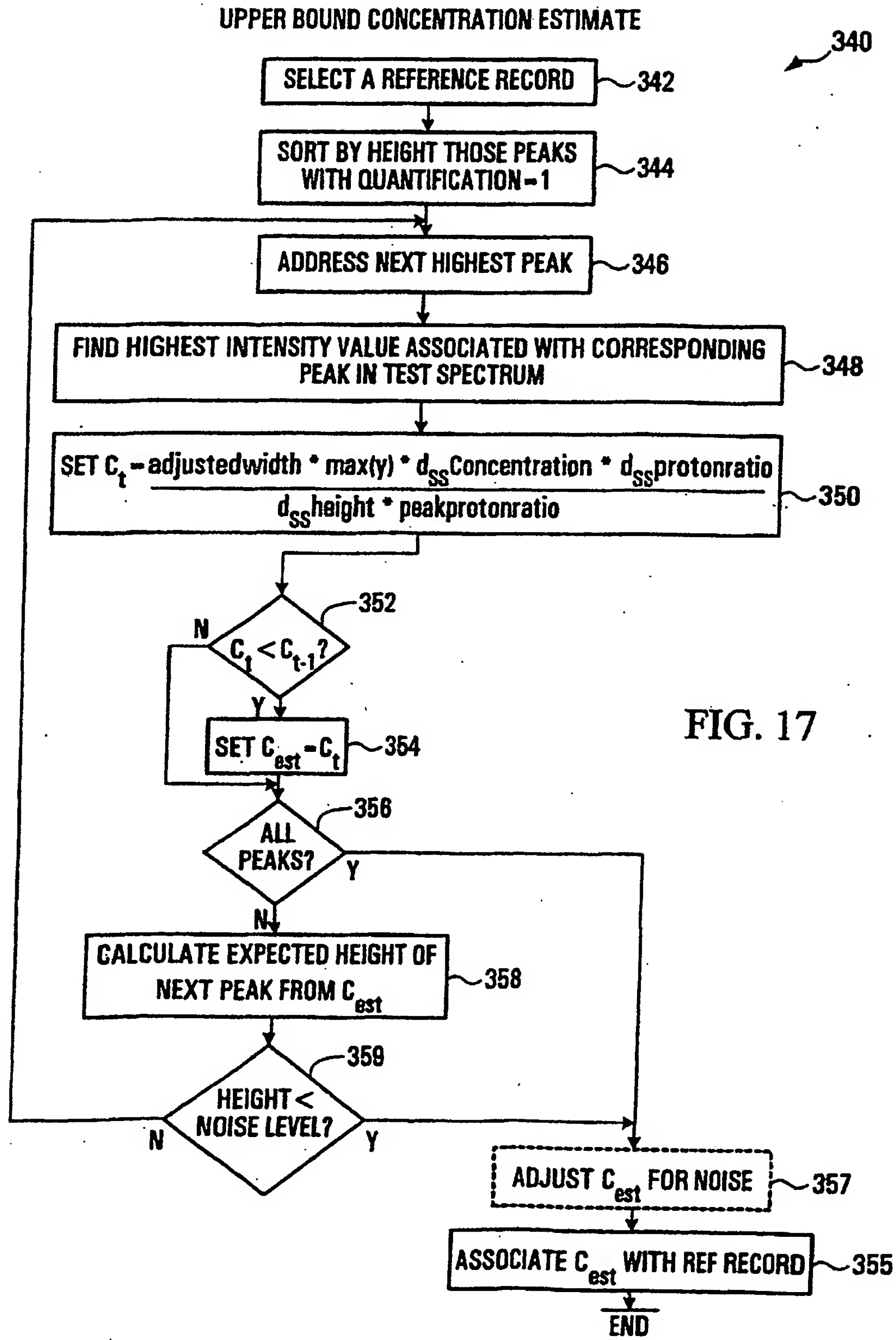

UPPER BOUND CONCENTRATION ESTIMATE
340
SELECT A REFERENCE RECORD
342
SORT BY HEIGHT THOSE PEAKS WITH QUANTIFICATION=1
344
ADDRESS NEXT HIGHEST PEAK
346
FIND HIGHEST INTENSITY VALUE ASSOCIATED WITH CORRESPONDING PEAK IN TEST SPECTRUM
348
SET $C_t = \frac{\text{adjustedwidth} * \max(y) * d_{SS}\text{Concentration} * d_{SS}\text{protonratio}}{d_{SS}\text{height} * \text{peakprotonratio}}$
350
$C_t < C_{t-1}$?
352
N
Y
SET $C_{est} = C_t$
354
ALL PEAKS?
356
Y
N
CALCULATE EXPECTED HEIGHT OF NEXT PEAK FROM C_{est}
358
HEIGHT < NOISE LEVEL?
359
N
Y
ADJUST C_{est} FOR NOISE
357
ASSOCIATE C_{est} WITH REF RECORD
355
END

FIG. 17

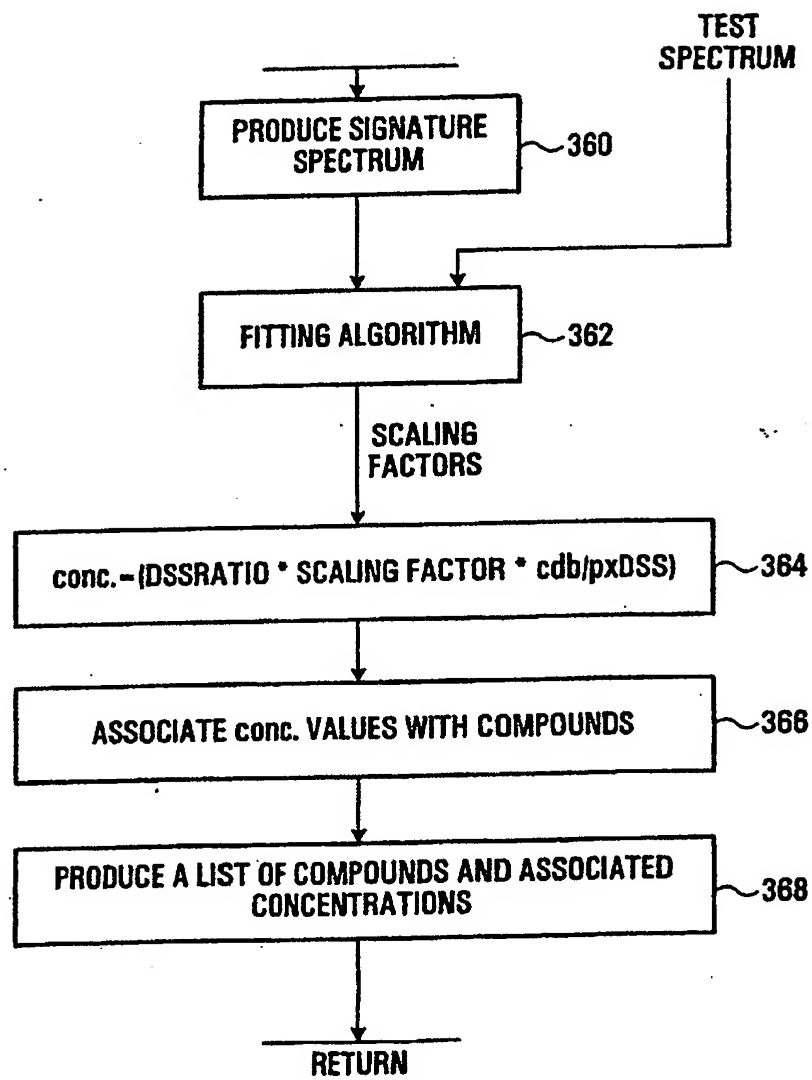

TEST SPECTRUM
PRODUCE SIGNATURE SPECTRUM
360
FITTING ALGORITHM
362
SCALING FACTORS
conc.=(DSSRATIO * SCALING FACTOR * cdb/pxDSS)
364
ASSOCIATE conc. VALUES WITH COMPOUNDS
366
PRODUCE A LIST OF COMPOUNDS AND ASSOCIATED CONCENTRATIONS
368
RETURN

FIG. 18

2410559

PROCESS, APPARATUS, MEDIA AND SIGNALS FOR AUTOMATICALLY IDENTIFYING COMPOUNDS IN A SAMPLE

BACKGROUND OF THE INVENTION

1. Field of Invention

This invention relates to qualitative and quantitative chemical analysis, and more particularly to processes, apparatus, media and signals for automatically identifying compounds in a sample.

2. Description of Related Art

The field of biometric identification has grown tremendously over the recent decade both from its relevance to medical diagnostics and to its application as a way to uniquely identify a person or an animal, for example. As diagnostic tools have become more sophisticated, complex liquid mixtures, such as human blood or urine for example, can now be analyzed to identify or search for particular compounds that can provide important diagnostic information to a medical technician or a doctor.

Generally, the separation and characterization of mixtures is fundamental to nearly every aspect of analytical chemistry and biochemistry. Most approaches to identify and quantify biological compounds in liquid mixtures require an initial compound separation (chromatographic or physical separation) step to separate a particular compound or set of compounds from the mixture. For example, gas chromatography, electrophoresis, and liquid chromatography are used to separate pure chemical components/compounds, for example, from a mixture before analysis is performed. Initial compound separation is required because most spectral identification processes, such as mass spectrometry or infrared, visible, and ultraviolet spectroscopy, require relatively pure samples in order to minimize

noise and increase the accuracy of the measuring device. Spectral identification processes are expensive, manually intensive and require a great deal of technical expertise to be performed properly in an accurate, timely manner.

Nuclear magnetic resonance (NMR) has recently been shown to be an alternative approach to identify and quantify biological compounds without chromatographic separation. In this approach, radio frequency (RF) electromagnetic radiation is applied to a mixture of organic compounds to extract and measure a characteristic RF absorption spectrum of nuclei belonging to each specific organic compound. A large number of compounds are associated with well-defined peaks in the absorption spectrum and knowing which peaks are associated with certain compounds makes it possible to manually identify some of the compounds in the liquid mixture without resorting first to chromatographic separation. However, this process is still quite slow and requires a great deal of a priori information that relates each peak to a given compound. It can take a number of years for experts in NMR spectroscopy to acquire the knowledge required to analyze NMR spectra to accurately identify and quantify compounds in sample mixtures.

Therefore what is desired is a process and apparatus for quickly, accurately and automatically identifying a number of compounds which may be present in complex liquid mixtures without involving chromatographic separation and without requiring people who are experts in NMR techniques.

SUMMARY OF THE INVENTION

<u>Overall Process</u>

The embodiments of the invention disclosed herein provide for automated, accurate analysis of a test spectrum obtained from a sample, to quantitatively and qualitatively identify compounds present in the sample.

In accordance with one aspect of the invention there is provided a process for producing a trace file for use in spectrum analysis, the process involving performing a Fourier Transform on Free Induction Decay (FID) data to produce an initial spectrum, filtering a selected region of the initial spectrum to produce a filtered spectrum, and phasing the filtered spectrum to produce a measured spectrum having a flat baseline and positive peaks.

In accordance with another aspect of the invention there is provided a computer readable medium for providing codes operable to direct a processor circuit to produce a trace file for use in spectrum analysis, the computer readable medium comprising codes for automatically causing the processor circuit to perform the process described above.

In accordance with another aspect of the invention there is provided an apparatus for producing a trace file for use in spectrum analysis, the apparatus comprising means for automatically performing a Fourier Transform on Free Induction Decay (FID) data to produce an initial spectrum, means for automatically filtering a selected region of the initial spectrum to produce a filtered spectrum, and means for automatically phasing the filtered spectrum to produce a measured spectrum having a flat baseline and positive peaks.

In accordance with another aspect of the invention there is provided a signal for causing a processor circuit to produce a trace file for use in spectrum analysis, the signal including a first segment comprising codes for automatically causing the processor circuit to perform the process described above.

In accordance with another aspect of the invention there is provided a computer-implemented process for producing a representation of a spectrum for a hypothetical solution containing a compound, for use in determining the composition of a test sample, the process involving producing a position value for at least one peak of a base reference spectrum as a function of a

measured condition of the test sample, and a property of the at least one peak in a base reference spectrum.

In accordance with another aspect of the invention there is provided a computer-readable medium for providing computer readable instructions for causing a processor circuit to produce a representation of a spectrum for a hypothetical solution containing a compound, for use in determining the composition of a test sample, the instructions comprising a set of codes for directing the processor circuit to perform the computer-implemented process described above.

In accordance with another aspect of the invention there is provided a signal operable to cause a processor circuit to produce a representation of a spectrum for a hypothetical solution containing a compound, for use in determining the composition of a test sample, the signal comprising a signal segment comprising codes operable to cause the processor circuit to perform the computer-implemented process as described above.

In accordance with another aspect of the invention there is provided an apparatus for producing a representation of a spectrum for a hypothetical solution containing a compound, for use in determining the composition of a test sample, the apparatus comprising a processor circuit programmed to produce a position value for at least one peak of a reference spectrum as a function of a measured condition of the test sample, and a property of the at least one peak in a base reference spectrum.

In accordance with another embodiment, there is provided an apparatus for producing a representation of a spectrum for a hypothetical solution containing a compound, for use in determining the composition of a test sample, the apparatus comprising means for receiving a measured condition value representing a measured condition of the test sample, means for receiving a representation of a position of at least one peak in a base reference spectrum, and means for producing a position value for at least one

peak of a derived reference spectrum as a function of the measured condition of the test sample, and the position of the at least one peak in a base reference spectrum.

Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying Figures.

BRIEF DESCRIPTION OF THE DRAWINGS

In drawings which illustrate embodiments of the invention,

Figure 1 is a system for determining the quantity of compounds in a test sample, according to a first embodiment of the invention;

Figure 2 is a flow chart illustrating an automatic process for conditioning a measured spectrum, as implemented by a workstation shown in Figure 1;

Figure 3 is a pictorial representation of a measured spectrum produced by the workstation shown in Figure 1;

Figure 4 is a flow chart of a routine executed on the workstation shown in Figure 1, for conditioning the measured spectrum to suppress a peak caused by a solvent in a sample for which the measured spectrum is produced;

Figure 5 is a flow chart of a process for identifying compounds executed by a spectrum analysis apparatus shown in Figure 1;

Figure 6 is a pictorial representation of a reference spectrum associated with lactic acid at pH of 5.10;

Figures 7A and 7B are a tabular representation of an Extensible Markup Language (XML) file representation of the reference spectrum of Figure 6;

Figure 8 is a flow chart of a process by which base reference spectrum records such as shown in Figures 7A and 7B may be produced;

Figure 9 is a process executed by the spectrum analysis apparatus shown in Figure 1 to identify a peak associated with a calibration compound in a test spectrum;

Figures **10A** and **10B** are a flow chart of the process for identifying compounds, shown in Figure **5**, in greater detail;

Figure **11** is a flow chart of a process executed by the spectrum analysis apparatus for determining a pH value from the test spectrum;

Figure **12** is a flow chart of a process executed by the spectrum analysis apparatus for producing a derived reference spectrum;

Figures **13A** and **13B** are a tabular representation of a base reference spectrum record associated with lactic acid at a pH of **5.45**;

Figures **14A** and **14B** are a tabular representation of a derived reference spectrum record associated with lactic acid at a pH of **5.28**;

Figures **15A** and **15B** are a tabular representation of a generic type of derived reference record in which equations specify center Parts Per Million (PPM) values for peak clusters, according to one embodiment of the invention;

Figures **16A** and **16B** are a tabular representation of a derived record comprising look-up table links to center PPM values according to another embodiment of the invention;

Figure **17** is a flow chart of a process for determining an upper bound concentration estimate;

Figure **18** is a flow chart of a least squares fitting routine referenced by Figure **10B**;

DETAILED DESCRIPTION

Referring to Figure **1**, a system, according to a first embodiment of the invention, for determining the quantity of compounds in a test sample is shown generally at **10**. The system includes a spectrum producing apparatus **12** and a spectrum analysis apparatus shown generally at **14**. In this embodiment, the spectrum producing apparatus **12** is a Nuclear Magnetic Resonance (NMR) System provided by Varian (RTM) Inc. of California, U.S.A. Generally, the system is operable to receive a specially prepared liquid biological test sample and produce a data file comprised of a plurality of (x,y) values which define a measured NMR spectrum. This measured NMR spectrum is then supplied to the spectrum analysis apparatus **14**, where a process according to another aspect of the invention is carried out to provide an indication of the quantities of certain compounds in the specially prepared biological test sample.

The system **10** is suitable for use with biological samples, for example blood or urine, in which the solvent is water, for example. Such samples may be "prepared" by doping them with a small quantity of a condition indicator compound, also referred to as a condition reference compound, and a chemically inert chemical shift calibration standard compound also referred to as a calibration compound. The condition indicator may be trimethylsilyl-1-propanoic acid or Imidazole, where the distortion factor is pH, for example. Alternatively, the sample itself may have a naturally occurring, inherent condition indicator such as glycine, creatinine, urea, citrate, or trimethylamine-N-oxide, for example. The chemical shift calibration standard compound may be 3-[trimethylsilyl]-1-propanesulfonic acid, also known as DSS, for example. Alternatively, the chemical shift calibration standard may be dimethylsulphoxide (DMSO), acetone, or tetramethylsilane (TMS), for example.

In this embodiment, the spectrum producing apparatus **12** is comprised of a computer workstation **16**, an auto sampler **18**, a test chamber **20**, and a

console **22**. The workstation is a Sun Workstation with a **400** MHz UltraSPARC IIi CPU with **2** MB level **2** cache, **128** MB RAM, on-board PGX**24** graphics controller, **20** GB **7200** r.p.m. EIDE hard disk **48**x CD-ROM drive **1.44** MB floppy drive and **17"** flat screen color monitor. The workstation runs Varian (RTM) VNMR software which includes routines for controlling the auto sampler **18** and the console **22** to cause the specially prepared biological liquid sample to be received in the test chamber **20** and to cause the console to acquire and provide to the workstation Free Induction Decay (FID) data representing the free induction decay of electromagnetic radiation absorptions produced by protons in the compounds of the liquid sample as a result of changes in magnetic properties of the protons due to a nuclear magnetic resonance process initiated in the test chamber **20** by the console **22**.

Process for Producing a Measured Spectrum

The FID data is received and stored in memory at the workstation **16**. Then, in this embodiment, a process according to an embodiment of another aspect of the invention, is carried out to cause the workstation to produce a measured spectrum for use by the spectrum analysis apparatus **14**. Instructions for directing the workstation to automatically carry out the process for producing the measured spectrum are embodied in computer readable codes **24**. These computer readable codes **24** may be provided to the workstation **16** in a variety of different forms including a file or files on a computer readable medium such as a CD-ROM **26**, or floppy disk **28**, for example, or as a file received as a signal from a communications medium such as an internet **30**, extranet or intranet, electrical **32**, Radio Frequency (RF) **34**, or optical medium **36** or any other medium by which a file comprised of said codes may be provided to the workstation **16** to enable the workstation to be directed by the codes to execute the process described herein to produce a measured spectrum.

Autoprocessing

Generally, an automatic computer-implemented process for producing a measured spectrum from NMR data, may involve operating on free induction decay (FID) data produced by a spectrometer to produce a trace file comprised of intensity and frequency values representing a measured spectrum having a flat baseline and well defined peaks that have positive, well-defined areas, for use in a computer-implemented spectrum analysis process such as the process described herein. In particular, the process may involve performing a Fourier Transform on Free Induction Decay (FID) data to produce an initial spectrum, filtering a selected region of the initial spectrum to produce a filtered spectrum and phasing the filtered spectrum to produce a measured spectrum having a flat baseline and well defined positive peaks.

Referring to Figure **2**, a flowchart depicting functional blocks implemented by the codes to cause the workstation to execute a specific process for producing a measured spectrum is shown generally at **50**. The process begins with a first block **52** that causes the workstation **16** to read and perform an initial Weighted Fourier Transform on the FID data to produce an initial measured spectrum representing signal intensity (i) versus frequency (F).

Then block **54** causes the workstation **16** to produce parameters for use in a later-executed Fourier Transform performed on the FID data to produce a representation of a measured spectrum having well defined Lorentzian lines with a flat baseline and peaks that have positive, well-defined areas. Thus, the result of block **54** is a set of parameters that controls Fourier Transforms later performed on the FID data to produce a representation of a measured spectrum.

Block **56** directs the workstation to save the set of parameters in association with the FID data. Block **58** directs the workstation **16** to perform a Fourier Transform on the FID data, using the parameters produced by block **54** to produce a trace file, which is a file comprised of a plurality of (x,y) values that

represent a trace of the measured spectrum, representing intensity versus frequency. Block **59** then causes the workstation to save the trace file for transmission to the spectrum analysis apparatus **14** shown in Figure **1**.

An example of a measured spectrum is shown generally at **41** in Figure **3**. The spectrum is a plot of intensity versus frequency. The x-axis **43** is referenced to parts per million (ppm) and depicts a window of the overall spectrum, the window containing relevant information or features for identifying compounds in the sample. The y-axis **39** is referenced to a zero value and the spectrum has a baseline **37** representing a noise level from which a plurality of peaks **45, 47, 49, 51, 53, 55, 57, 59, 61, 63** associated with various compounds in the sample extend. For example peaks **45** and **47** are associated with Imidazole, peak **49** is associated with Urea and peaks **51** and **53** are associated with Creatinine. Peaks **55** and **57** form a first cluster associated with citric acid and peaks **59** and **61** form a second cluster associated with that compound. Peak **63** is associated with DSS, the calibration compound.

Referring back to Figure **2**, block **54** which processes the FID data, is shown in greater detail. Block **54** includes sub-functional blocks including a Fourier Transform block **60**, a filter selected and/or solvent region block **66** and an automatic phasing block **68**, each of which is automatically executed in turn, in the order shown. The process may include an optional spectral window setting block **62** and an optional drift correction block **64**, to further process the spectrum, for example.

The Fourier Transform block **60** has an optional sub-block **70** that causes the workstation to perform a weighted Fourier Transform with weights that provide for enhancement of the initial spectrum. These weights may perform a line broadening function to the initial spectrum, for example. To do this in this embodiment, block **70** causes the workstation to set signal enhancement parameters for use in a subsequently executed weighted Fourier Transform

block **72**. Such signal enhancement parameters may effect line broadening, line narrowing, or gaussian sine-bell conditioning, for example, to the resulting spectrum produced by the Fourier Transform block **72**. In the Varian (RTM) VNMR software, this is effected by setting a line broadening variable "lb" to a specified value, which may be **0.5**, for example. Also in the VNMR software, the weighted Fourier Transform may be executed by calling the VNMR macro "wft" to perform a weighted Fourier Transform on the FID data, using the lb parameter value set at block **70**. This has the effect of broadening the lines or peaks of the spectrum and averaging the spectrum to produce a measured spectrum with a better signal to noise ratio than would be produced without averaging. It also has the effect of eliminating glitches to produce a measured spectrum of better quality.

In this embodiment optional block **62** causes the workstation **16** to define a window on the initial spectrum and this may involve scaling the initial spectrum. It is desirable to set the spectral window to a preset size, i.e. a pre-defined range of frequency, to enable the acquisition of repeatable data and for all useful data to be in a pre-defined window and to scale the spectrum such that the height of its maximum peak is a percentage of the height of the window. In this embodiment, this is effected through the VNMR software by executing three sub-functional blocks **74**, **76** and **78** that cause the workstation **16** to call the VNMR macros "f", "full', and the VNMR command "vsadj", respectively, in the order shown. The 'f' macro sets display parameters "sp" and "wp" for a full display of a 1D spectrum, the **'full'** macro sets display limits for a full screen so that the spectrum can be seen as wide as possible in the window, and the **'vsadj'** command sets up automatically the vertical scale "vs" in the absolute intensity mode "ai", so that the largest peak is of the required height. Effectively this provides for scaling of the spectrum so that the highest peak is **90%** of the total window height.

Optional block **64** causes the workstation to produce parameters that perform drift correction on the spectrum to correct the measured spectrum for drift

effects, effectively setting the two extremes of the baseline of the spectrum, i.e. the left and right sides of the spectrum to have zero slope. In this embodiment, using the Varian (RTM) VNMR software, this is achieved by block **80** which causes the workstation **16** to call the "dc" macro of the VNMR software. Effectively the "dc" macro calculates a linear baseline correction. The beginning and end of a straight line to be used for baseline correction are determined from the display parameters "sp" and "wp". The "dc" command applies this correction to the spectrum and stores the definition of the straight line in the parameters "lvl" (level) and "tlt" (tilt) of the VNMR software. (cdc resets the parameters "lvl" and "tlt" to zero.)

Block **66** causes the workstation to filter a selected region of the spectrum to adjust the intensity of the spectrum in that region. Filtering may involve applying a notch filter to a selected or solvent region, for example, to suppress a peak associated with a contaminant or solvent in the contaminant or solvent region. This ensures that the solvent region or contaminant region of the spectrum is correctly phased with the rest of the spectrum so that the entire spectrum can be properly phased later. In order to permit the entire spectrum to be phased, the solvent or contaminant residual must be in phase with the rest of the spectrum, ideally reducing the solvent or contaminant region to zero. The solvent region is the region of the spectrum in which solvent compounds in the sample may be found. For example the solvent may be water, in which case the region around the peak in the measured spectrum associated with the compound H_2O is considered to be the solvent region. The contaminant region is a region of the spectrum where peaks associated with contaminants are present.

Referring to Figure **4**, a routine for filtering the selected region is shown generally at **66** and involves a first block **92** that causes the workstation **16** to apply a notch filter to the selected region to suppress a peak in that region. A set of initial notch filter parameters specifying the attenuation, width and position of the notch filter is used.

Applying a notch filter may further involve producing an adjusted set of notch filter parameters and applying a notch filter employing the adjusted set of notch filter parameters to the selected region. The set of notch filter parameters may be adjusted to produce an adjusted set of notch filter parameters that may be applied to the notch filter to filter the selected region until a sum of the absolute values of areas defined by peaks above and below a baseline of the initial spectrum is minimized. In this embodiment this is done by block **94** which causes the workstation to adjust the set of initial notch filter parameters and re-apply the notch filter until the sum of the absolute values of the areas of the spectrum in the selected region, is minimized. One quick way of doing this and minimizing the number of iterations of application of the notch filter is to employ numerical methods to successive values produced. For example, in this embodiment, using the Varian (RTM) VNMR software, the parameter "sslsfrq" specifies a notch filter value that affects the minimization of the sum of the areas above and below the baseline. Brent's method, as described in Brent, R.P. 1973, Algorithms for Minimization without Derivatives (Englewood Cliffs, NJ: Prentice-Hall), Chapter 5, [1], for example may be used to find an optimum value for "sslsfrq".

Referring back to Figure 2, after filtering the selected region block **68** is invoked to automatically phase the entire spectrum and make the peaks as symmetrical as possible. This may be done iteratively, for example, by adjusting the real and imaginary components of the transformed FID data until the resulting spectrum has positive, well defined peaks. In this embodiment, employing the Varian (RTM) VNMR software, this is achieved by invoking block **84** which calls the "aph0" command of the VNMR software. Some versions of the VNMR software may require more than one successive execution of the *aph0 command.*

After automatic phasing parameters of the spectrum have been produced, optionally, a baseline correction block **69** may be executed to flatten out the baseline of the spectrum. Alternatively, baseline correction may be performed

later. Baseline correction may be done by analysing the spectrum to determine areas with peaks and areas devoid of peaks and setting areas devoid of peaks to have a common intensity value such as zero, for example. An example of baseline correction available at www.acdlabs.com/publish/nmr_ar.html published by Advanced Chemistry Development Inc. of Toronto, Ontario, Canada.

Block **56** then causes the workstation **16** to save parameters produced by the various sub-processes of block **54** in association with the FID data and text, if desired. With the Varian (RTM) VNMR software this may be achieved using the **'svf($savefid)'** command.

Block **58** then directs the workstation **16** to produce a trace file comprised of (x,y) values representing intensity versus frequency, by performing a Fourier Transform on the FID data, using the parameters produced as described above and associated with FID data. The trace file is then transferred or transmitted to the spectrum analysis apparatus **14** or is stored for later transfer to that apparatus.

Spectrum Analysis Apparatus

In the embodiment shown, the spectrum analysis apparatus (SAA) **14** is a separate component and includes a Linux workstation configured to receive the trace file representing the measured spectrum, from the spectrum producing apparatus **12**. The spectrum analysis apparatus **14** is configured to receive and execute instructions embodied in computer readable codes to carry out a process for identifying compounds in a sample according to an embodiment of another aspect of the invention. The codes may be provided to the spectrum analysis apparatus through any of the media described above including the CD-ROM **26**, Floppy disk **28**, internet **30**, extranet, intranet, electrical **32**, RF **34**, and optical **36** media and/or any other media capable of providing codes to the spectrum analysis apparatus.

It will be appreciated that the workstation **16** may alternatively be configured with both the codes to effect the process for producing a measured spectrum shown in Figure **2** and the codes to effect the process for identifying compounds, or either of these. It is desirable however, to execute the process for identifying compounds at a computer other than the workstation **16**, to enable the process for identifying compounds to be executed while another sample is being subjected to the NMR process, for example.

<u>Process for Identifying Compounds</u>

Referring to Figure **5**, generally, the process for identifying compounds involves identifying representative reference spectra from a set of reference spectra associated with detectable compounds and selected according to a condition of the sample, which collectively define a composite reference spectrum having features matching a set of features in a test spectrum produced from the sample. Once the representative reference spectra have been identified, compounds with which they are associated may be identified.

The compounds associated with respective reference spectra of the identified set are the compounds that may be expected to be present in the sample. Quantities of the compounds may be determined from the intensities of certain representative peaks in the test spectrum which are associated with the compounds, relative to the intensity of a peak associated with the chemical shift calibration standard compound which is unaffected by the condition of the sample. A condition may be the pH of the sample, for example, and an accurate measurement of pH can be obtained from the test spectrum. Thus, given a test spectrum of a sample and given a set of reference spectra, the process can identify and quantify compounds present in the sample. Alternatively, the condition may be temperature, osmality, salt concentration, chemical composition, or solvent, for example.

Reference Spectra

Before the process for identifying compounds can be carried out, a set of reference spectra for compounds to be detected in the sample must be made available to the SAA **14**. This can be done by storing data relating to reference spectra associated with respective compounds and allowing the SAA **14** access to the data. An exemplary reference spectrum for a given compound may initially be represented in the form of intensity versus frequency (x,y) values, which may be represented graphically. A reference spectrum for lactic acid is shown in Figure **6**, for example. It will be appreciated that such a spectrum may have a plurality of peaks and/or clusters of peaks **150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170** superimposed upon a featureless background, such as noise **172**. The resolution along the x axis is dependent upon the frequency of the Magnet used in the Nuclear Magnetic Resonance Process employed to acquire the sample. The peaks that are associated with lactic acid are found in first and second clusters **166** and **154**. These clusters are centered at **1.322** ppm and **4.119** ppm respectively. The first cluster is comprised of two peaks and the second cluster **154** is comprised of four peaks.

A reference spectrum of the type shown in Figure **6** can be represented in various formats including mathematical representations such as Lorentzian equations which may specify peaks associated with the compound the spectrum is intended to represent. Such equations have the form:

$$f(x) = \frac{a w^2}{w^2 + 4(x-c)^2}$$

where:

where:	a	*represents amplitude of the peak*
	w	represents width of the peak; and
	c	represents the center of the peak

Thus, for example, the two peaks associated with the cluster centered on **1.322** ppm may be specified by two sets of Lorentzian line shape parameters a, w and c.

The Lorentzian line shape parameters for each peak associated with a given compound may be stored in a base reference spectrum record embodied in an XML file as shown in Figures **7A and 7B**, for example. Such a file may have fields **200, 202** and **204**, for example, for storing compound information, experiment information and cluster/peak information respectively. The compound information field may include sub-fields for storing the name of the compound with which the record is associated, and the molecular weight of the compound, for example. The experiment field may have sub-fields for storing information about the experiment, such as conditions under which the peak information about the compound was collected. This may include the pH of the solution that was analyzed, the temperature of the solution, the calibration reference compound ratio, the concentration of the compound in the solution, a timestamp, a sourcefile name, the frequency of the magnet used in the NMR process, and the spectral width of the entire spectrum, for example. The cluster/peak information fields may include separate fields **206** and **208** for each cluster (**166** and **154** in Figure **6**).

Each cluster field **206** and **208** may include sub fields **210, 212, 214, 216** and **218** for representing information relating to the proton number of the cluster, the quantification of the cluster, the Lorentzian line width adjustment of the cluster and first and second peak subfields respectively. The first and second peak subfields may include fields **220, 222**, and **224** for representing offset center information, height information and proton ratio information relating to a respective peak in the cluster, respectively.

Effectively, the Lorentzian line shape parameters (a) and (c) for each peak may be stored in the height and offset center fields **222** and **220** respectively and each peak in a given cluster is considered to have the same width (w)

which is specified by the contents of the Lorentzian line width adjust field **214** associated with the cluster.

Referring to Figure **8**, a process by which base reference spectrum records may be produced is shown generally at **230**. The process begins with block **232** representing the preparation of a liquid solution containing a reference compound such as lactic acid, a calibration compound such as DSS and a condition indicator compound such as Imidazole. The liquid solution is prepared to a carefully calibrated concentration of the calibration compound at a carefully controlled temperature and pH. This step is carried out in a laboratory, by a human or by a mechanized process, for example.

Once the liquid solution containing the reference compound has been produced, as shown in block **234**, it is subjected to the NMR process carried out by the apparatus **12** shown in Figure **1** to produce FID data.

At block **236**, the apparatus **12** subjects the FID data produced by the NMR process to the process shown in Figure **2**, to produce a measured reference spectrum.

Having obtained a measured reference spectrum, a process as shown in block **238** is initiated to identify the calibration compound and obtain calibration parameters. This process is shown in greater detail at **238** in Figure **9**. Referring to Figure **9**, the codes direct the SAA **14** to derive from the measured reference spectrum a characterization of the calibration compound contained in the sample. This involves identifying a position of a peak of the measured reference spectrum that meets a set of criteria that associate the peak with the calibration compound and further involves producing parameters for a mathematical model of the peak, that best represents the peak. Thus, in this embodiment the characterization is a list of Lorentzian line shape parameters (w, c and a) representing width, peak position and center amplitude respectively of a Lorentzian curve that best

describes a feature, that is, a peak, of the measured reference spectrum, that is associated with the calibration compound. It will be appreciated that other characterizations could be used, such as those produced by peak picking, linear least squares fitting, the Levenberg-Marquardt method, or a combination of these methods.

To find a peak associated with the calibration compound and to produce a list of Lorentzian line shape parameters that characterize it, the SAA **14** is programmed with codes that include a first block **250** that directs the SAA **14** to determine a noise level at a pre-defined area of the measured reference spectrum. In this embodiment, it is known that an area on the x-axis (frequency) corresponding to positions **64,000** and **65,000** for example can be expected to be void of peaks and contain only noise. The standard deviation of the y-value (signal intensity) over this region of the measured reference spectrum is representative of the noise level of the entire spectrum and provides a measure of the noise level.

Next block **252** directs the SAA **14** to scan the measured reference spectrum in the negative x-direction beginning at the higher order end of the spectrum, to find a y-value that meets a certain criterion. For example, the criterion may be that the y-value must exceed the noise level by a pre-determined amount, such as a factor of **10**, at the top of a peak. A y-value meeting this criterion is assumed to be associated with an x-value that represents the position of a peak associated with the calibration compound.

Block **254** then directs the SAA **14** to employ the x-value representing the approximate position of the calibration peak in the test spectrum in a fitting algorithm that fits a curve to the calibration peak and specifies width, height and position values. For example a Lorentzian line shape-fitting algorithm may be employed to produce Lorentzian line shape parameters (a, w and c) that define a Lorentzian line shape that best matches the calibration peak.

Referring back to Figure **8**, having calculated Lorentzian line shape parameters that identify and characterize the calibration compound, block **240** is carried out to associate other input data with the measured reference spectrum. Other input data may include information associated with the name and experiment fields **200** and **202** and information such as the number of protons (proton number in XML file) for each cluster and the proton ratio for each peak, for example.

Next at block **242**, the measured reference spectrum is characterized by employing the well-known Conjugate Gradient method to determine Lorentzian line shape parameters (a, w and c) for each peak or to determine sets of such parameters that define a mathematical model or models of peaks that best fits the important peaks of the measured reference spectrum.

At block **244**, a base reference spectrum record of the type shown in Figures 7A and 7B is produced from the other input data and the characterization of the spectrum. At block **246**, the base reference spectrum record is stored in a reference record library, which effectively includes a plurality of reference records for various different reference compounds. For example, the reference record library may include base reference spectrum records for L-Phenylalanine, L-Threonine, Glucose, Citric Acid, Creatinine, Dimethylamine, Glycine, Hippuric Acid, L-Alanine, L-Histidine, L-Lactic Acid, L-Lysine, L-Serine, Taurine, Trimethylamine, Trimethylamine-N-Oxide, Urea, L-Valine, and Acetone.

Reference records may include base reference records or derived reference records. Base reference spectrum records may be produced by empirical processes as described above. New records known as derived reference records may be produced by operating on data from base reference records, and represent derived reference spectra. Operating on data may include interpolation and/or performing mathematical operations, and/or using a lookup table, for example. Thus, for example, a limited set of base reference

spectrum records can be produced, including a record representing the spectrum for lactic acid at a pH of 5.1, and a record for lactic acid at a pH of 5.45, for example. A derived reference record representing the spectrum of lactic acid in a solution having a pH of 5.28, for example, can then be produced by performing mathematical operations on the Lorentzian line shape parameters specified by the base records associated with solutions at pH 5.1 and pH 5.45 to interpolate values for a solution at a pH of 5.28. Thus, a derived set of reference records can be produced for solutions of any pH, within a reasonable range, when required, thereby avoiding a priori production of base reference records for every pH condition. As will be appreciated below, this feature may be exploited by determining the pH value of a sample under test and using the determined pH value to produce a set of derived reference records for use in identifying compounds present in the sample. In other words, reference records for use in the process for identifying and quantifying compounds are selected from existing base reference records or are "selected" by producing derived reference records, according to a condition of the sample. In this embodiment, the condition is pH.

Process for Identifying Compounds

After having produced a reference library of base reference spectrum records, the process of identifying and quantifying compounds in a test sample can be carried out.

Process for Identifying and Qualifying Compounds

The process is shown generally at **300** in Figure **10A** and **10B** and begins with an optional first block of codes **302** that cause the SAA to perform a spectrum conditioning step.

Spectrum Conditioning

If the measured NMR spectrum of the test sample is of sufficient quality, it can be used directly in subsequent operations of the process disclosed herein. However, usually, the measured spectrum will not be of sufficient quality and will require further processing to condition it for later use. This further conditioning may involve baseline correction as described earlier, for example, to produce a conditioned spectrum.

Thus the following description will refer to a test spectrum, which may be the measured spectrum described above, if such measured spectrum is of sufficient quality or it may be a conditioned spectrum. A measured spectrum having a corrected baseline, for example, would be an example of a measured spectrum that would not need to be subjected to further processing to condition it. Usually, however, the process will involve producing a test spectrum from the measured spectrum.

Calibration Determination

After being provided with, or after producing, a test spectrum of the type described, the process involves block **304** to produce a characterization of a calibration compound in the sample or block **306** to determine a representation of a condition of the sample. These two functions can be done independently or the determination of the condition of the sample can be determined after first characterizing the calibration compound.

The process of characterizing the calibration compound generally involves identifying a peak associated with a calibration compound, in the test spectrum. This may involve identifying a peak meeting a set of criteria that

associate the peak with the calibration compound. The peak associated with the calibration compound may be characterized by producing Lorentzian line shape parameters to represent the peak.

Block **304** relating to characterizing the calibration compound involves a call to the process shown in Figure **9** to cause the SAA **14** to produce a set of Lorentzian values (a, w and c) which best represent the peak associated with the calibration compound in the test spectrum.

Condition Factor Determination

Optionally, as shown by block **308**, a separate measuring device may be used to measure the selected condition of the test sample. In this embodiment, the measured condition is pH which may be measured by a separate pH meter to produce a pH condition value that may be supplied to the SAA as indicated at "C" in Figure **10**, for use in later functions of the process.

If the condition value has not already been obtained desirably the condition value can be derived from the test spectrum itself as shown at block **306**. This is possible where the measured condition is pH because the identification of a peak associated with a pH indicator compound in a sample can be readily determined from the test spectrum and the Lorentzian line shape values that characterize the representation of the calibration compound in the test spectrum.

Referring to Figure **11**, a process for determining a pH condition value from the test spectrum is shown generally at **310**. Basically, the process involves identifying a position, height and width of a peak associated with a condition reference compound in the test spectrum and this may involve identifying a peak meeting a set of criteria that associate the peak with the condition reference compound. Once the peak is identified the measured condition value may be produced as a function of the peak position and parameters of

the sample medium, the parameters being the parameters that define the calibration compound.

To achieve this, in this embodiment, the codes include a block **312** which directs the SAA **14** to employ the Lorentzian line shape parameter (c) associated with the calibration compound to locate a window in the test spectrum, where a peak associated with the pH indicator compound is expected to be. The window is then scanned along the x-axis (frequency) from left to right, for example, for a y-value (intensity) that is greater than the amplitude value specified by the Lorentzian line shape parameter (a).

When a y-value meeting the above criteria is found, block **314** causes the SAA 14 to execute a characterization algorithm to produce at least a center value (c) representing the center of the peak associated with the pH reference compound. For example a Lorentzian curve algorithm may be used to produce Lorentzian parameters a, w and c defining the peak associated with the pH reference compound.

Block **316** then directs the SAA **14** to execute a modified pH titration Equation as shown below, on the center value c and to use certain parameters of the sample solvent, in the equation, to produce a condition value representing pH of the sample:

$$pH = pK_A - \log\left[\frac{\delta_{obs} - \delta_A}{\delta_{HA} - \delta_{obs}}\right]$$

Where: δ_{obs} is the observed chemical shift (center c);
δ_A is the chemical shift of the conjugate base;
δ_{HA} is the chemical shift of the conjugate acid; and
pK_A is an association constant for the conjugate base.

Assume that no matter what method of determining pH is used, a pH value of **5.28** is obtained for the sample. Referring Back to Figure **10B** block **320** directs the SAA to receive the condition value either produced externally, such as by measurement or produced internally such as by using the test spectrum as described above, to produce a derived reference record representing a derived reference spectrum for use in later functions of the process. Separate derived reference records may be produced from corresponding base reference spectrum records associated with corresponding compounds expected to be in the sample. Thus, in effect a representation of a set of derived reference spectra may be produced from a set of reference spectra and the measured condition value. In general, a process for producing a representation of a spectrum for a hypothetical solution containing a compound, for use in determining the composition of a test sample, involves producing a position value for at least one peak of a reference spectrum as a function of the measured condition of the test sample and a property of the at least one peak in a base reference spectrum. The property may be a position of a peak, amplitude of the peak or width of the peak for example. In this embodiment, a derived reference record is used to represent a representation of a spectrum for the hypothetical solution.

Referring to Figure **12**, producing a derived reference record may involve accessing a pre-defined record specifying peaks in a reference spectrum and adjusting a position value in the record, the position value being the position value of the at least one peak. This may be done by block **322** which causes the SAA to identify a base reference spectrum record that is associated with a condition nearest to the measured condition of the sample and to use such reference spectrum as the derived reference spectrum.

Producing a position value for a peak may involve interpolating a position value from position values associated with base reference spectra associated with condition values above and below the measured condition value associated with the sample. For example, block **324** may be employed to

cause the SAA **14** to produce a position value by calculating the position value as a function of pH of the sample and to effectively produce or interpolate a derived reference spectrum.

To interpolate a derived reference spectrum, assume that at block **322** a base reference record for lactic acid at a pH of **5.10** is located as being the base reference spectrum record for lactic acid that is nearest to the pH of the sample, **5.28**. Such a record is shown in Figures **7A** and **7B**. Referring back to Figure **12**, block **324** may direct the SAA **14** to find another base reference spectrum record for lactic acid that is associated with a pH value greater than the pH of the sample. Assume that it locates a base reference spectrum record associated lactic acid at a pH of **5.45**. A record of this type is shown in Figures **13A** and **13B**. On locating this second base reference spectrum record, block **324** directs the SAA **14** to create a new derived reference spectrum record for lactic acid at a pH of **5.28**. To do this the SAA **14** is directed to make a copy of the base reference spectrum record associated with a pH of **5.45** and then to replace the frequency values for the center position of each cluster shown in that record with interpolated values. A simple linear interpolation is used to find the value **1.3202** for the first cluster and the value **4.1149** for the second cluster. Figures **14A** and **14B** show the resulting derived reference spectrum record for a pH of **5.28**, for lactic acid, produced using this method. Similarly, derived reference spectrum records are produced for each compound in the reference library to produce derived reference records for a pH of **5.28** for each compound represented in the library.

Alternatively, adjusting the position of a peak may involve locating a measured condition value dependent function in a base reference record, or pre-defined record, producing the position value from the function and associating the position value with the pre-defined record. Associating may involve storing the position value in the pre-defined record, for example. To effect this method of adjusting the position of a peak, a generic type of derived

record may be kept, in which equations, effectively specifying the centerPPM values for the two clusters as a function of pH may be provided in the field associated with the centerPPM value for each cluster, as shown in Figures **15A** and **15B**. Then, whenever a pH value is found from a sample, a copy of the record can be made and the pH value may be used in the equations in the copied record to produce centerPPM values. These centerPPM values can then be substituted for the respective equations that produced the, in the copied record, thereby producing a new derived record for use in later calculations.

Alternatively, producing a position value may involve producing the position value by addressing a lookup table of position values with the measured condition value of the sample. For example the position value of a peak may be adjusted by locating, in a pre-defined record, a link to a lookup table specifying peak positions for various condition values, retrieving the position value from the lookup table and associating the position value with the pre-defined record. To do this a second generic type of derived record may be kept, in which lookup table links, effectively specifying links to lookup tables (not shown) that return centerPPM values for input pH values may be provided in the field associated with the centerPPM value for each cluster, as shown in Figures 16A and 16B. Then, whenever a pH value is found from a sample, a copy of the record can be made and the pH value may be used to address the lookup tables associated with the links specified in the record to produce centerPPM values. These centerPPM values can then be substituted for the respective links that produced them, in the copied record, thereby producing a new derived record for use in later calculations.

Referring back to Figure 10B, after having produced a derived reference spectrum for each compound that is likely to be in the sample, block **326** causes the SAA **14** to calibrate the Lorentzian line width values for the derived reference spectrum relative to the test spectrum to provide for a better fit to the test spectrum. To do this, block **326** may direct the SAA **14** to calibrate to

the (a, c and w) values associated with the calibration compound in the sample, the spectral linewidths of peaks associated with each of the reference compounds. In this embodiment block **326** may direct the SAA **14** to employ the contents of the Lorentzian width adjust field **214** of each derived reference spectrum record to produce respective absolute values representing actual linewidths relative to the calibration compound linewidth. These modified spectral line widths may be associated with respective peaks in the same cluster of each reference compound, by storing these modified spectral line widths in an internal data structure (not shown) that associates modified spectral information with derived reference records.

Still referring to Figure **10B**, optionally, compound specific adjustments as shown by block **328** may be made to the contents of the fields of the derived reference records, where it is known, for example that certain effects occur when certain reference compounds are present in the test sample. For example, the shift of peaks associated with citrate is affected by the presence or absence of certain divalent cations and therefore the process may include a compound-specific adjustment to compensate for shifts known to occur when the presence of such divalent cations is known. Other compound-specific adjustments may be made to compensate for shifts due to temperature, chemical interactions, dilution effect and other ligand effects.

Cluster Centering

Still referring to Figure **10B** the process may further involve a cluster centering step as shown at **330** for shifting the derived reference spectrum in frequency (x-direction) to better align it with the test spectrum. This may involve producing a cluster position indicator for a derived reference spectrum, which causes the positions of peaks in the derived reference spectrum to match corresponding peaks in the test spectrum. A cluster position indicator already associated with the derived reference spectrum may be used or a cluster position indicator that produces a match of the derived reference spectrum to the test spectrum to a defined degree may be derived from the cluster position

indicator already associated with the derived reference spectrum. In the embodiment shown, producing a cluster center indicator is achieved by attempting to fit the cluster to the test spectrum. To do this, cluster center values around the cluster center value already associated with the derived reference spectrum are assigned to the derived reference spectrum and used to effectively shift the derived reference spectrum to the left and right of the current cluster center value. For example, cluster center values +/- **0.001** ppm points are successively assigned to the derived reference spectrum to successively shift the center of the derived reference spectrum at successive points in a window extending **-0.003** ppm to **+0.003** ppm from the currently assigned cluster center. At each point, the derived reference spectrum is used in a Levenberg-Marquardt (LM) fitting algorithm that determines a correlation value for each position of the center of the derived reference spectrum in the window. The center position that causes the LM fitting algorithm to produce the best correlation value is then associated with the derived reference spectrum correlation value and is used in later calculations. Thus in effect, the derived reference spectrum is "wiggled" into alignment with the test spectrum. This wiggling is done independently for each cluster of peaks in the derived reference spectrum.

<u>Upper Bound Concentration Estimates</u>

Still referring to Figure **10B**, in this embodiment, the process for identifying and quantifying further involves block **332** which causes the SAA **14** to produce an upper bound estimate of a quantity of a compound associated with a derived reference spectrum, for use in a least squares algorithm later in the process. In general, producing an upper bound concentration estimate comprises selecting as the upper bound concentration estimate, a lowest concentration value selected from a plurality of concentration values calculated from respective peaks in the test spectrum. This may involve finding the height of a peak in the test spectrum that corresponds to a peak in the reference spectrum and determining a concentration value for the peak as a function of its height. Prior to determining a concentration estimate for a

peak, the process may involve predicting whether the height of a peak in the test spectrum is greater than a threshold level and deciding not to determine a concentration for the peak when the height is less than the threshold level.

Referring to Figure **17** a process implemented by program codes operating on the SAA **14** of Figure **1**, for producing an upper bound concentration estimate is shown generally at **340**. A first block **342** causes the SAA **14** to select a reference record. Next block **344** causes the SAA **14** to sort by height those peaks in the reference record that have a quantification value equal to **1**. This causes the process to consider only those peaks that provide reliable concentration estimates. Next, block **346** directs the SAA to address the (next) highest peak of those that have just been sorted at block **344**. Reference is made to the "next" high peak because the peaks are considered in succession. On the first pass through the process however, the highest peak found in the sort is the first peak addressed.

Next block **348** causes the SAA **14** to use the position of the currently addressed peak in the reference spectrum to locate a corresponding peak in the test spectrum. This may involve looking for a peak in a window positioned at a corresponding position in the test spectrum. On finding such a peak, the maximum intensity value (max(y)) associated with that peak is found.

At block **350**, the SAA **14** is directed to calculate a concentration value as a function of the max (y) value, using the following equation:

$$Ct = \frac{\text{adjustedwidth} * \max(y) * \text{dssconcentration} * \text{dssprotonratio}}{\text{Dssheight} * \text{peakprotonratio}} \qquad \textbf{(17)}$$

Where:	Ct	is the concentration value for the peak
	adjustedwidth	is the width of the peak as determined from the variable w calculated as shown in

	Figure **9** and the Lorentzian width adjust value stored in the reference record
max(y)	is the maximum y-value associated with the corresponding peak in the test spectrum
dssconcentration	is the concentration of DSS in the sample 0.5mM, for example
dssprotonratio	is the DSS proton ratio (9, for example)
Dssheight	is the DSS height value a, calculated as shown in Figure 9
Peakprotonratio	is the proton ratio of the peak, as indicated in the reference record.

At block **352** SAA **14** is directed to determine whether the currently calculated concentration value is less than the previously calculated value. If so, then block **354** causes the SAA **14** to set a preliminary upper bound concentration value to the current concentration value. If at block **352**, the currently calculated concentration value is not less than the previously calculated value, the preliminary upper bound concentration estimate value remains at its former value. The effect of blocks **352** and **354** is to cause the preliminary upper bound concentration estimate to be set to the lowest concentration value calculated for any of the peaks.

Once the preliminary value has been determined from the current pass, block **356** directs the SAA **14** to determine whether all peaks with quantification values of **1** have been considered. If so, the SAA **14** is directed to optional block **357** in Figure **17**. If not, the SAA **14** is directed to block **358** which causes the SAA to calculate the expected height of the next peak associated with the compound, in the test spectrum. To do this equation **17** above is solved for max(y) using the current preliminary concentration estimate, and the Lorentzian width adjust value, and the peak proton ratio of the next highest peak from the list of sorted peaks. Then, block **359** in Figure **17** causes the SAA **14** to determine whether the max(y) value so found is less

than the noise level of the spectrum. (noise level was calculated at block **250** in Figure **9**). If not, then the next peak is worth considering and the SAA **14** is directed to resume processing at block **346** to address the next highest peak in the sorted list.

If the estimated height of the next highest peak found at block **358** is less than the noise level of the spectrum, the SAA **14** is directed to an optional block **357** which increases the amplitude of the preliminary concentration estimate value by the amplitude of the noise in the test spectrum to produce a true estimate of the upper bound concentration limit for the compound. This is useful where concentration values are very low.

Then, finally, block **355** directs the SAA **14** to associate the true upper bound concentration estimate with the reference record, such as by storing the upper bound concentration estimate value in a field (not shown) of the record, or in a field of a data structure maintained in the SAA **14** to create such associations.

Least squares Fitting

Referring back to Figure **10B**, the process for identifying and quantifying compounds involves a block **334** which causes the SAA **14** to perform a least squares fitting algorithm using all of the derived reference records and the test spectrum to produce scaling values for each peak in each reference spectrum such that when all peaks from all reference spectra are summed they produce a composite spectrum that best matches the test spectrum.

Referring to Figure **18**, the least squares fitting routine includes a first block **360** which causes the SAA **14** to produce "signature" spectra comprised of (x,y) pairs that define a composite spectrum representative of the sum of all Lorentzians in a given derived reference record. A separate signature spectrum is produced for each derived reference record. Thus a separate (x,y) array is produced for each derived reference record.

Block **362** then provides each signature spectrum, upper bound concentrations and the (x,y) array representing the test spectrum to a Linear Least Squares fitting routine, which in this embodiment is LS SOL licensed from Stanford University of California, USA. This routine returns scaling factors for each peak in each applicable reference record, such that when the scaled Lorentzian models specified in all applicable reference records are summed together to make a composite spectrum, the composite spectrum has features matching features in the test spectrum produced from the sample. These scaling factors thus identify representative reference spectra from a set of reference spectra associated with detectable compounds and selected according to the measured condition of the sample.

In this embodiment, an indication of compounds associated with reference spectra having peaks that when scaled by the scaling factors have a height greater than a threshold may be produced. This may involve producing a list of compounds, for example. Thus, scaled peaks having a height less than the threshold may indicate that the presence of the associated compound in the sample is questionable and therefore such compound should not be listed as being present in the sample.

Block **364** then causes the SAA **14** to employ these scaling factors in the following equation to quantify each compound by producing concentration values for each compound represented by a reference record:

Conc. = (DSSRatio * scalingFactor * cdb) / pxDSS

Where:	Conc.:	concentration of the given compound in the sample
	DSSRatio:	the DSSRatio entry for the given compound (see field **202** in Figure **7A**)
	scalingFactor:	the scaling factor of the highest peak in the given compound (from least squares fitting)

cdb:	the concentration of the given database entry (see field **202** in Figure **7A**)
pxDSS:	the pixel height of DSS in the spectrum (the value a as determined by the process shown in Figure **9**)

Block **366** then causes the SAA **14** to associate these concentration values with the compounds associated with the derived reference records.

Block **368** then causes the SAA **14** to produce a list or indication of compounds in the sample, along with their associated concentration values. This list may be printed and/or displayed on a monitor, for example. Concentration values may be expressed in moles, mmol/L, g/L or moles/mole, for example and absolute quantities may be obtained by a simple equation converting concentration to absolute quantity values, in moles, for example.

While specific embodiments of the invention have been described and illustrated, such embodiments should be considered illustrative of the invention only and not as limiting the invention as construed in accordance with the accompanying claims.

CLAIMS

1. A computer-implemented process for producing a trace file for use in spectrum analysis, the method comprising:

   performing a Fourier Transform on Free Induction Decay (FID) data to produce an initial spectrum;

   filtering a selected region of said initial spectrum to produce a filtered spectrum; and

   phasing said filtered spectrum to produce a measured spectrum having a flat baseline and positive peaks,

   wherein performing a Fourier transform comprises performing a weighted Fourier Transform with weights that provide for enhancement of said initial spectrum.

2. The method of claim 1 wherein filtering comprises applying a notch filter to said selected region to suppress a peak associated with a contaminant in said contaminant region.

3. The method of claim 2 wherein applying a notch filter comprises producing an adjusted set of notch filter parameters and applying a notch filter employing said adjusted set of notch filter parameters to said selected region.

4. The method of claim 3 wherein applying a notch filter comprises iteratively adjusting said set of notch filter parameters and applying said adjusted notch filter parameters to a notch filter and applying said notch filter to said selected region until a sum of the absolute values of